Journal of
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Vol. 41 JULY, 1958 NUMBER 7

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The Eleventh Annual Convention of the National Association of Artificial Breeders will be held at New Orleans, Louisiana, August 17–20, 1958. All A.D.S.A. members are welcome.

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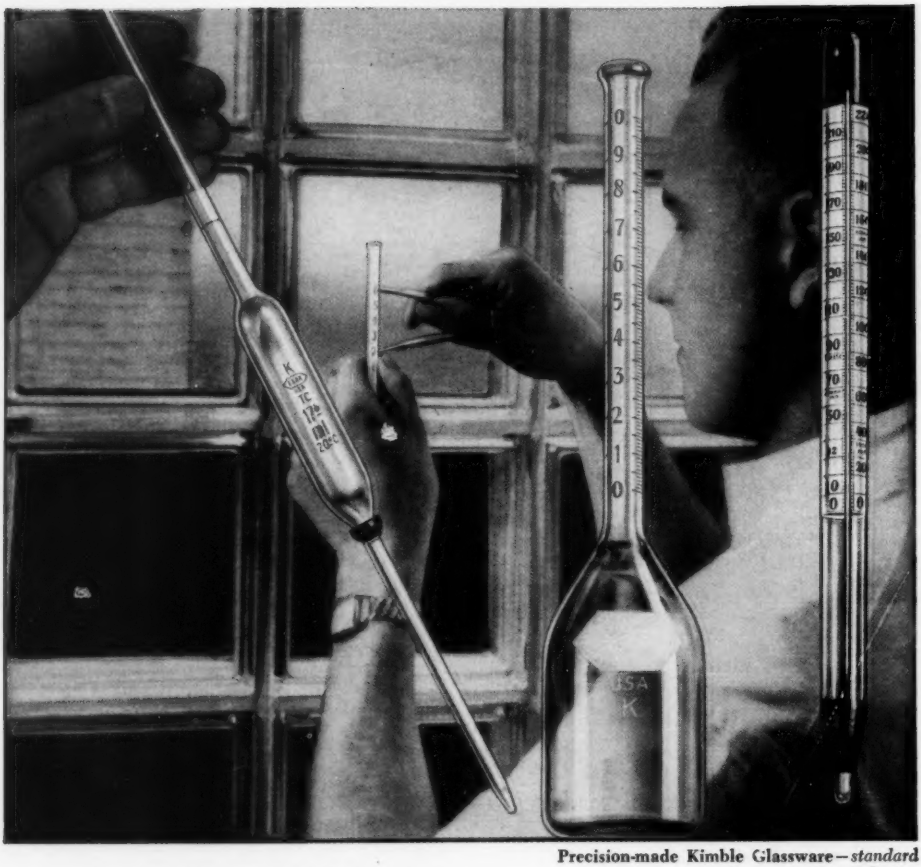

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PEOPLE AND EVENTS

Recent Deaths

L. R. BRYANT, dairy chemist and professor of dairy chemistry, Ontario Agricultural College, died May 10, 1958.

Widely known in the dairy science field both in Canada and the United States, "Len" Bryant was born in England in 1898 and received his B.A. and M.A. degrees from the Univ. of Toronto. He was appointed to the Ontario Agricultural College faculty in 1927 and was made dairy chemist in 1934. Prof. Bryant taught senior courses in dairy and physical chemistry and graduate courses in the physical chemistry of dairy products.

L. R. Bryant

Prof. Bryant was the author or coauthor of many research papers in the dairy and food research field. Some of his research work included studies on can- and bottle-washing, boiler waters, sugar beet analyses, oxidative rancidity of fats, freezing points and S.N.F. content of Ontario milk, and aluminum milk cans.

He was a past president of the Hamilton Chemical Association, a past member of the Council of the Canadian Institute of Chemistry, and a past president of the Wellington-Waterloo branch of the Chemical Institute of Canada. In 1935, Prof. Bryant was elected a Fellow of the Canadian Institute of Chemistry.

W. H. RIDDELL, 61, chairman of the Dairy Husbandry Dept., Univ. of Vermont, died June 27, 1958, at Burlington.

Born in Fort Langley, B.C., Canada, Dr. Riddell received his B.S. degree from the Univ. of British Columbia in 1922 and his M.S. and Ph.D. degrees from the Univ. of Minnesota in 1924 and 1932, respectively.

During World War I he served as a private in the Canadian Army in England, 1917 to 1919. In World War II, he was nutrition officer at Walter Reed Hospital, Washington, D.C., and later was the first post-war agricultural attaché in the Netherlands, serving under Supreme Headquarters of the Allied Expeditionary Forces with the rank of Lieutenant Colonel. For his services, the Dutch government decorated him with the order of Orange-Nassau, rank of Commander.

Before going to the Univ. of Vermont, Dr. Riddell was engaged in teaching and research at Kansas State College and was head of the Dairy Science Dept. at the Univ. of Arizona.

He was a member of the A.D.S.A., the American Society of Animal Production, and Sigma Xi. In his community, he was active in the Rotary Club, the College Street Congregational Church, and the Burlington Community YMCA.

Survivors include his widow; a son, William H., Jr., U.S. Information Service in Cambodia; a daughter, Mrs. Martha Wetzel, Tulsa, Okla., and his mother.

Completed Theses

M.S. Degree

E. C. CORNER—Effectiveness of various vacuum, temperature, and steam treatments in reducing feed flavors in milk. Kansas State College, Manhattan.

A. G. HUNTER—Investigations on the etiology of bovine glandular vulvovaginitis. Univ. of Rhode Island, Kingston.

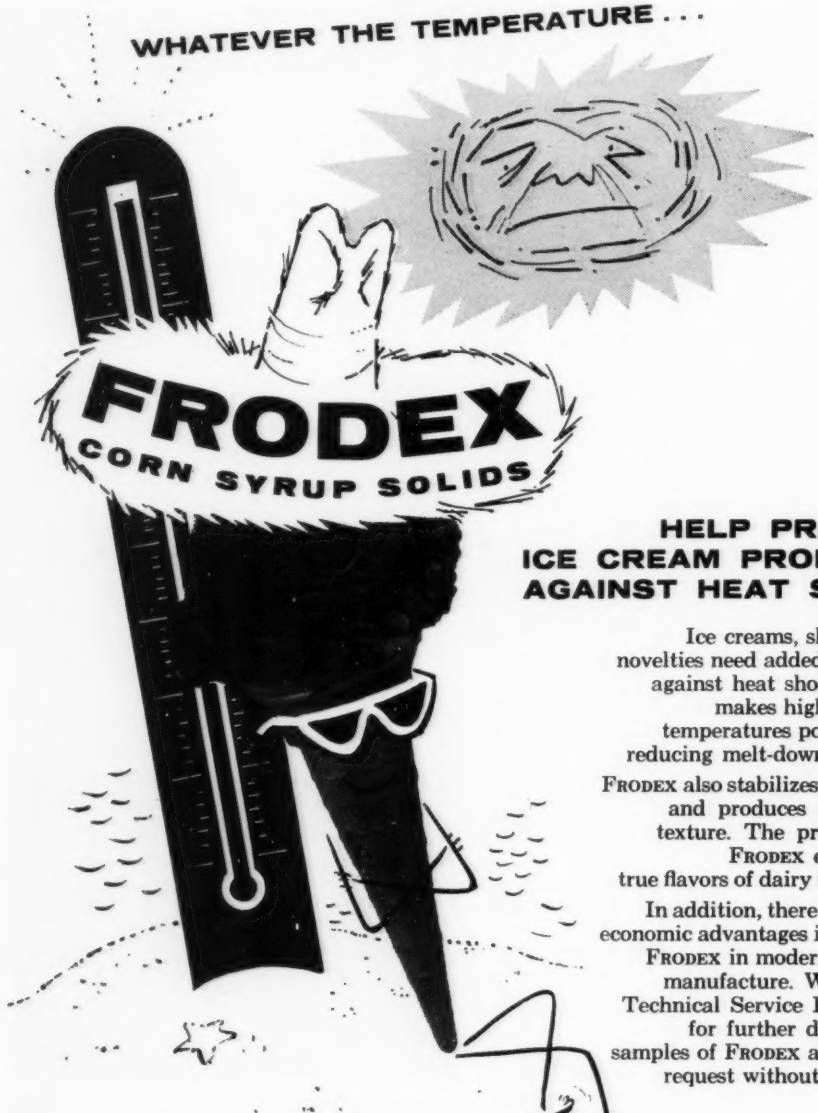
J. F. VAN TILBURG—Effects of selected proteolytic bacteria on Cheddar cheese ripening. Kansas State College, Manhattan.

Tennessee News

The Tennessee Dairy Products Association has contributed $1,500 in scholarships to the Univ. of Tennessee for 1958. The check was given to N. D. PEACOCK, dean of the College of Agriculture, by B. V. LAWSON, secretary of the Tennessee Products Association. The gift was made at a banquet of the Dairy Club held in April.

Scene at the 1958 Univ. of Tennessee Dairy Club Banquet as B. V. Lawson, secretary of the Tennessee Dairy Products Association, hands a scholarship check to N. D. Peacock, dean of the College of Agriculture. Pictured (left to right) are Tom Young, president of the U. T. Dairy Club, Lawson, W. F. Moss, Tennessee Commissioner of Agriculture, and Dean Peacock.

The scholarships are to be awarded to three juniors and three seniors majoring in dairy manufacturing. This is the second year that such scholarships have been provided at the Univ. of Tennessee by this Association.



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A. G. VAN HORN, superintendent of the Dairy Experiment Station at Lewisburg, Tenn., retired in July after more than 20 yr. as a dairy husbandman with the USDA. A group of friends, relatives, and coworkers honored Mr. Van Horn with a dinner.

Mr. Van Horn became superintendent of the station in 1939. Before that, he had served 2 yr. as a county agricultural agent in Atchison, Kan. He then operated a purebred Holstein farm for 9 yr. Later, he was a dairy agent with the USDA at Woodward, Okla. This work preceded his transfer to Lewisburg, where the USDA has acquired land for a new dairy experiment station. The station is now operated jointly by the Univ. of Tennessee and the USDA.

C. A. HUTTON, dairy analyst of the University Agricultural Extension Service and longtime dairy extension specialist, retired in July.

C. A. Hutton

Mr. Hutton graduated from the U.T. College of Agriculture in 1913. He was the first dairy extension specialist appointed in Tennessee, in 1914, and served in that capacity until 1948.

Mr. Hutton has aided in the development of the dairy industry in Tennessee in countless acts of service. Among them are the guidance of cooperative creameries and cheese factories, the initiation of D.H.I.A. organizations, providing aid to dairy marketing cooperatives, and the organization of four federated artificial breeding associations.

Kansas News

RICHARD BASSETTE has been appointed to the staff of the Dept. of Dairy Husbandry, Kansas State College, as assistant professor of dairy manufacturing. He replaces W. D. RUTZ, who left the staff in February to work with Fairmont Foods Co., Omaha, Neb.

Dr. Bassette received his Ph.D. degree from the Univ. of Maryland, College Park, this year. He will teach and do research in the field of dairy manufacturing.

The Ninth Annual Nutrition School for Feedmen, to be held Aug. 18–19 at the Univ. of Wisconsin, Madison, will have as one of its participants, E. E. BARTLEY, professor of dairy husbandry. Dr. Bartley will give a paper on the subject of "Antibiotics for Dairy Cattle."

Errata

The May issue of the Journal carried two research papers in which significant typographical errors occurred. The references to the papers and corrections follow:

L. J. Teply *et al. J. Dairy Sci.*, **41**: 593. 1958.
Table 6, page 602:
First Column, third line, should read "Total Gain ..... 123.6".
First Column, fourth line, should read "Total Feed Consumption ..... 457.7".
First column, fifth line, should read "Protein Efficiency ..... 3.04".
Reference No. 7 should read "*Dairy Sci. Abstracts*, **18**: 364. 1956."

D. H. Bullock and W. C. Winder, *J. Dairy Sci.*, **41**: 708. 1958.
Line 13, column one, should read . . . "the initial sinkability of the fresh powder could not only be retained but could be improved considerably."

N.A.A.B. To Meet at New Orleans

The Eleventh Annual Convention of the National Association of Artificial Breeders will be held Aug. 17–20, 1958, at the Jung Hotel, New Orleans, La.

High lights of the four-day program include two important panel discussions, a banquet address, and two other major talks.

One panel discussion will be on the topic, "Ways of Eliminating the Scrub Bull," with B. J. BURCH, JR., Hammond, La., as moderator. Participants will be E.A. WENNER, extension dairyman, Michigan State Univ., East Lansing; R. J. SMITH, breeding technician, L.A.B.C., New Iberia, La.; E. W. NEASHAM, extension dairyman, and P. I. HIGHLEY, general manager, American Breeders' Service.

The other panel discussion will be on "Procuring, Training, and Supervising Technicians," with CHARLES KRUMM, Southern Wisconsin Breeding Cooperative, as moderator. Participants will be F. G. STEVENSON, M.F.A. Dairy Breeders; VINCENT HINKLEY, New York Artificial Breeders Cooperative, Ithaca; E. N. HANSEN, Curtiss Improved Stud Service, Cary, Ill., and W. H. ARMSTRONG, Virginia Artificial Breeding Association, Rocky Mount, Va.

Banquet speaker will be Dr. KENNETH MCFARLAND, Topeka, Kan., educational consultant and lecturer for the General Motors Corporation. Other addresses will be "What Is the Business Outlook Today?" by R.W. ELSASSER, business counselor, New Orleans, La., and "New Equipment Used in Sire Analysis" by J.F. KENDRICK, D.H.I.A. and Sire Proving Division, USDA.

West Virginia News

Some 250 persons attended the Ninth Annual West Virginia Univ. Dairy Day held in June on the West Virginia Univ. Dairy Farm.

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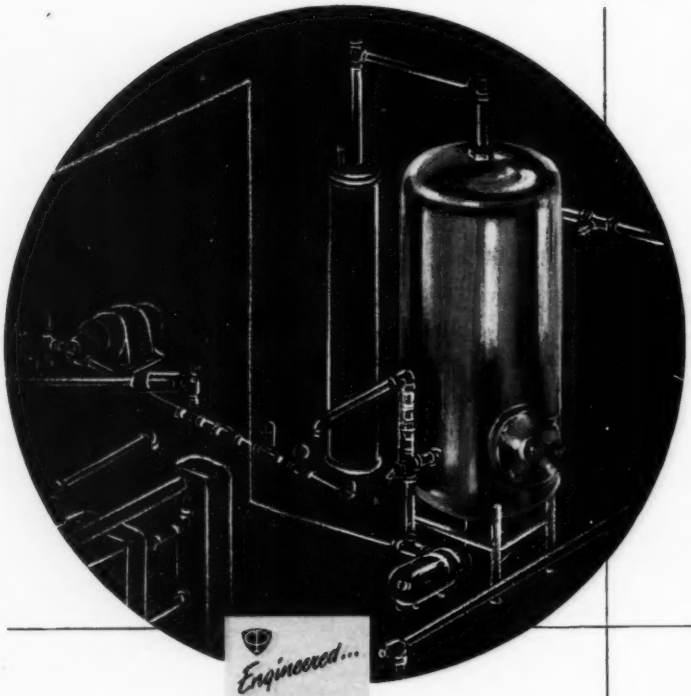

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Staff members of the Dairy Dept., the Agricultural Engineering Dept., and the Agronomy Dept. cooperated on the event. Main speaker was Dr. W. E. PETERSEN, Dairy Dept., Univ. of Minnesota.

Dr. Petersen spoke on the subject, "New Services the Dairy Cow Can Render."

Other phases of the program included a display of the newest developments and methods in hay- and silage-making, new developments in weed control, and inspection of four breeds of dairy cattle. I. D. PORTERFIELD was general chairman of the event.

V.P.I. News

J. P. GARBER, a junior student in the Dept. of Dairy Science, received the 1958 Ralston Purina undergraduate award of $500. The award is based on academic proficiency, need, and extracurricular activities.

M. F. ELLMORE of the Dept. of Dairy Science was elected to the Graduate Studies and Degrees committee of the Agricultural Faculty at its May meeting. G. C. GRAF, head of the Dept. of Dairy Science, was elected as the Agricultural Faculty representative on the V.P.I. Advisory Committee on Academic Freedom and Tenure at the same meeting.

The National Association of Artificial Breeders made a grant to the Virginia Agricultural Experimental Station for studies on dairy cattle breeding. The work will be done under the supervision of N. R. THOMPSON.

W. A. HARDISON appeared on a symposium on "Forage Quality and Its Evaluation," sponsored by the American Grassland Council and the American Dairy Science Association, at Raleigh, N. C., in June. The title of Dr. Hardison's talk was "Evaluating the Nutritive Quality of Forage on the Basis of Energy."

Montana News

LEE ADAMI, who graduated from Montana State College in June with a major in dairy manufacturing, has been awarded the Virginia Dare Award. This award is presented to senior students in dairy manufacturing by the Virginia Dare Extract Company of Brooklyn, N.Y. The award consisted of a check for $25 plus an engraved plaque.

The 23rd Annual Dairy Manufacturing short course will be held at Montana State College, Bozeman, Nov. 17–19, 1958.

The program consists of lectures on current dairy problems, demonstrations, and a dairy products show. Further information is available from the Dept. of Dairy Industry, Montana State College, Bozeman, Mont.

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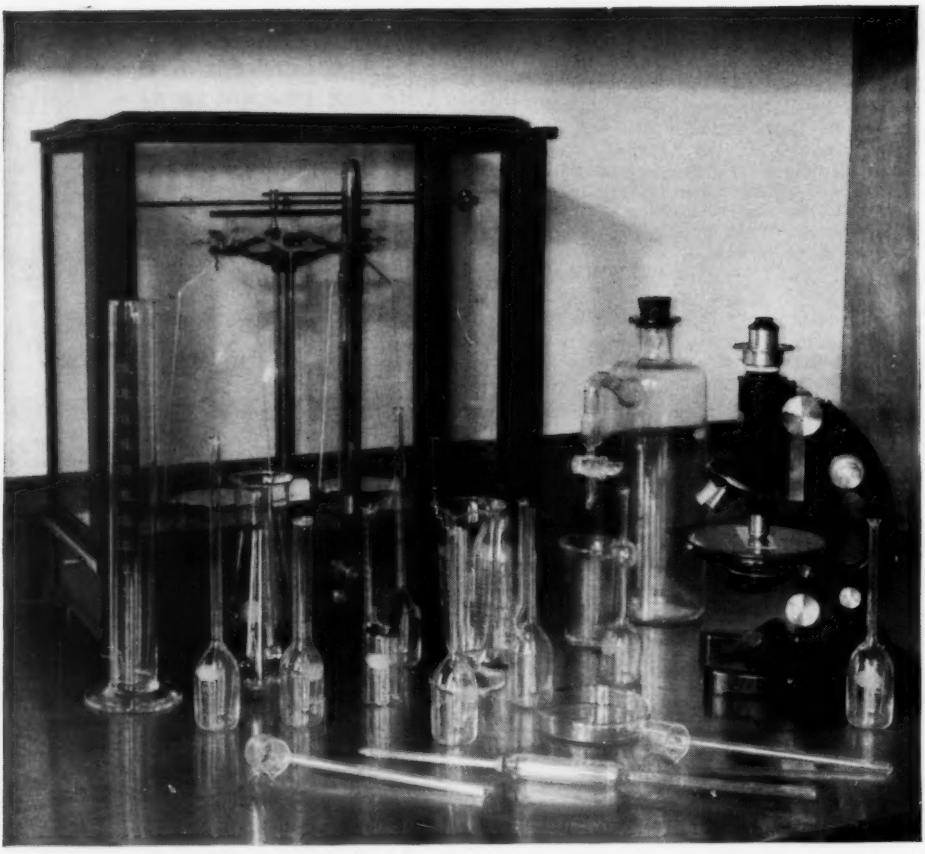

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FDA Appoints Detroit Chief

G. T. DAUGHTERS has been appointed Chief of the new Detroit District of the Food and Drug Administration. H. M. BOLLINGER was named Chief Chemist for the district.

The Detroit District is the Food and Drug Administration's 17th field headquarters operation and the first new district added to the agency's field organization in 24 yr. Office and laboratory facilities will be housed in a new, specially constructed building. The new district includes Michigan and the northern parts of Indiana and Ohio—an area now served by the Chicago and the Cincinnati Districts.

Mr. Daughters, who has been with the Food and Drug Administration for 30 yr., has directed the Chicago District for the past 3½ yr. Previously, he served as Chief of the Baltimore District. Mr. Bollinger has been Chief Chemist of the Los Angeles District since 1951.

Mr. Daughters began his FDA career as a chemist in the San Francisco District. He became a food and drug inspector at Salt Lake City in 1930, was promoted to Chief Inspector, and served at Denver, Los Angeles, San Francisco, New Orleans, and St. Louis. He was born in Davenport, Washington, and received his B.S. degree in chemistry from Whitman College, Walla Walla, in 1927.

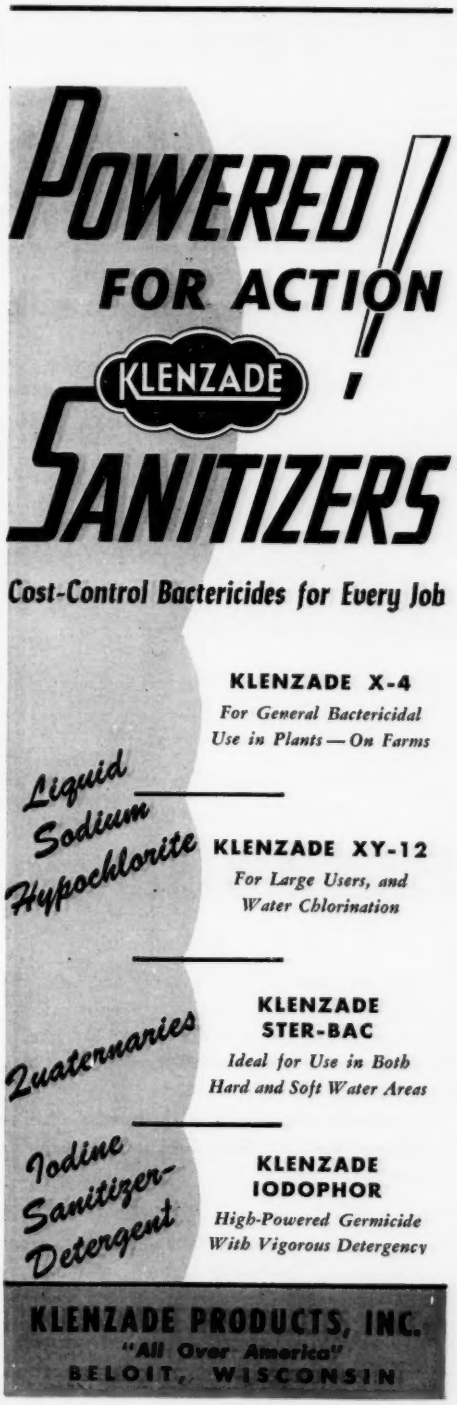
Mr. Bollinger has served his entire career in FDA's Los Angeles District, beginning as a chemist in 1936. He has made distinguished contributions to the development of analytical methods useful in food and drug law enforcement. He received his A.B. and M.A. degrees in chemistry from the Univ. of California at Los Angeles.

Wentworth Elected to S.A.R. Post

W. A. WENTWORTH, president of the International Dairy Show (Oct. 6–11 in Chicago), has been elected president-general of the National Society of The Sons of the American Revolution. Mr. Wentworth, a resident of Frankfort, Ky., was unanimously elected last month at the S.A.R.'s 68th Annual Congress in Biloxi, Miss.

A well-known figure in the dairy industry, Mr. Wentworth retired 2 yr. ago as Director of Industry Relations for the Borden Company. Industry posts held during his 27 yr. of service with Borden's include: Chairman of the Board, National Dairy Council; Chairman, Dairy Industry Committee; President, Dairy Products Improvement Institute, and President, Dairy Shrine Club.

The son of a former president-general of the S.A.R., who served two terms, 1916 and 1917, Mr. Wentworth joined the Iowa Society of the S.A.R. in 1911. He has received the S.A.R.'s Minute Man Award, and served in various national and state S.A.R. offices.



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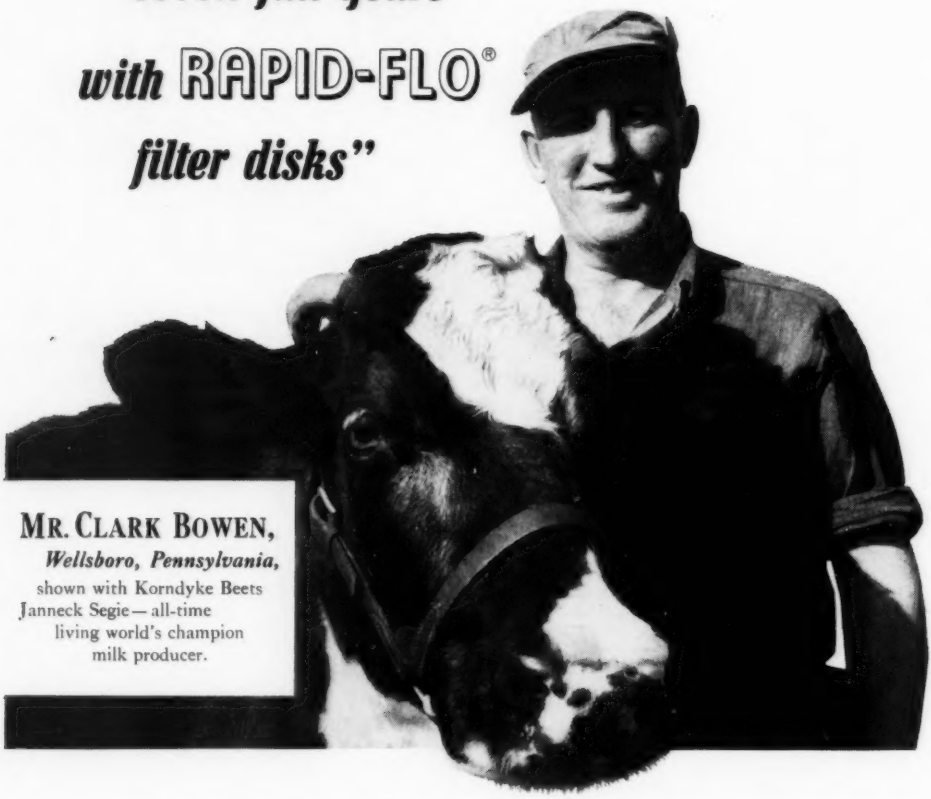

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Perry Returns from Lebanon

E. J. PERRY, New Brunswick, N. J., has recently returned from Lebanon, where he served as animal hubandry adviser with the International Cooperation Administration's mutual security program in this Middle East country. En route to the United States, he and his wife visited the Royal Agricultural and Veterinary College in Copenhagen, Denmark, and the Agriculture Division of the Univ. of Ghent in Belgium. Following consultation in Washington, D. C., Mr. Perry attended the annual A.D.S.A. meeting at North Carolina State College, Raleigh.

Arriving in Egypt in January, 1956, Mr. Perry assisted the Egyptian Government in setting up an animal husbandry improvement program, which he supervised until hostilities broke out in the Suez Canal zone on October 29, 1956. Evacuated from Cairo by the U. S. Sixth Fleet, Mr. Perry joined the American technical assistance mission in Lebanon, where he served for 16 mo. as the animal husbandry adviser to the Lebanese Government.

Concentrating chiefly on helping the Lebanese Ministry of Agriculture in lowering the cost of milk production and increasing the nation's supply of milk, Mr. Perry helped to set up a program designed to accomplish these objectives. Imported cattle and their progeny from Holland are presently providing from 30 to 50% more milk than similar milk cattle of Lebanon, while an artificial insemination program introduced in the country upon his recommendations has resulted in improving breeds throughout the nation. Working closely with the Director of the Agricultural Experiment Station at Terbol, Mr. Perry helped to establish a demonstration program in proper breeding management for the Lebanese Government. Besides introducing improved feeding practices, the New Brunswick agricultural expert produced a pamphlet on artificial insemination in the Arabic language for use by cattle-raisers in Lebanon. He also helped the Lebanese Government to produce three talkie films on animal husbandry, to be shown in the rural areas of the country.

Mr. Perry is one of many Americans, specialists in various fields, who are working in some 60 countries of the free world, helping to advance the objectives of ICA's partnership program.

Bulkley Speaks at California Polytechnic

California will continue to have fewer and fewer dairy farms, the industry has become definitely specialized, it's no longer profitable to operate "sideline herds," there's no place for "absentee management" in modern dairying, and operating efficiency will determine the dairymen who prosper and those who fail.

These were the views of George S. Bulkley, director of extension for the Carnation Company, and a speaker at the Thirty-Ninth Annual Convention of the California Agricultural Teachers Association held recently on the home campus of California State Polytechnic College. He spoke on "The Changing Dairy Industry" and reviewed the A.D.S.A. meeting just completed at North Carolina State College.

Some 400 agricultural teachers, representing high schools and junior colleges throughout the state, heard Bulkley stress good management practices as the key to modern dairy profit.

From the Secretary's Desk

Each year one-half of the ballots for officers are printed with one set of names at the top and on the other half the names are reversed.

This is on the theory that some may have a tendency to vote for the first name they come to.

This year, I was interested to find that one of the candidates for Vice-president got 51% of his total vote when *his* name appeared first on the list, while the other candidate got 56% of his total vote when his name appeared first on the list.

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STUDENT CHAPTER NEWS

J. E. JOHNSTON, *Editor*

Cal Poly Los Lecheros Dairy Club High Lights of the School Year

There were three outstanding high lights of the year. After three years of red tape and debate, Los Lecheros Dairy Club was permitted to put the first commercial milk-vending machine on campus. After 6 wk. of experience, the milk-vending machine has been popular beyond all expectations.

Another event which stood out very prominently in the year was the annual Dairy Club Banquet, which honored G. M. Drumm who has retired from teaching after 26 yr. as head of the Dairy Department. He is known all over the United States as a dairy cattle judge and for his development of good herds of three breeds of dairy cattle at Cal Poly.

The most outstanding event of the year has been the publication of the Los Lecheros Dairy Club Yearbook. It is thought to be the only strictly dairy publication of its kind west of Kansas State College. Because Cal Poly is a learn-by-doing school, the club members themselves have to learn by doing, by engaging in every aspect of this enterprise. The yearbook is a comprehensive survey of the Dairy Department and club activities. It tells what the students are doing to gain knowledge in our learn-by-doing system, featuring both dairy husbandry and dairy manufacturing activities. It also salutes the graduating seniors and includes their biographies. Copies are available on request.

Among the routine events are judging contests and a fitting and showing contest, sponsored by the Dairy Department at the annual open house during Cal Poly Royal. The Cal Poly team was third in the Inter-Collegiate Dairy Cattle Judging Contest and was first at the Grand National Livestock Exposition Collegiate Dairy Cattle Judging Contest. The products team was first at the Western Regional Dairy Products Judging Contest and was 15th at the Collegiate Student International Dairy Products Judging Contest.

The year has been very successful and rewarding, due to the cooperation and hard work of the Los Lecheros Dairy Club members.

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Georgia Student Chapter Holds Banquet

The dedication of the *Georgia Dairyman* was the high light of a banquet held in the Georgia Center for Continuing Education on May 16 by the Dairy Science Club. The speaker for the event was Mr. Charles Cullen, of the Cullen Sales Agency of Charlotte, North Carolina.

The 1958 *Georgia Dairyman*, the official annual of the Dairy Science Club, was dedicated to Miss Virginia Doster and Miss Marie Scott, both of Athens. Miss Scott and Miss Doster are associated with the Dairy Department of the University of Georgia. Their helping hand to students and everyone with whom they come in contact has won a place for each of them in the hearts of everyone they meet. Miss Doster and Miss Scott have been with the Dairy Department for several years, in the capacity of clerk and statistician.

Melvin Hoeflin, of Buffalo, New York, a senior in the Dairy Department, received a $1,000 scholarship from the Southern Association of Ice Cream Manufacturers. Melvin was picked from competition open to all seniors from all southern universities.

Richard Peper was the recipient of the Swift and Company Essay Award, which included an expenses-paid trip to Chicago.

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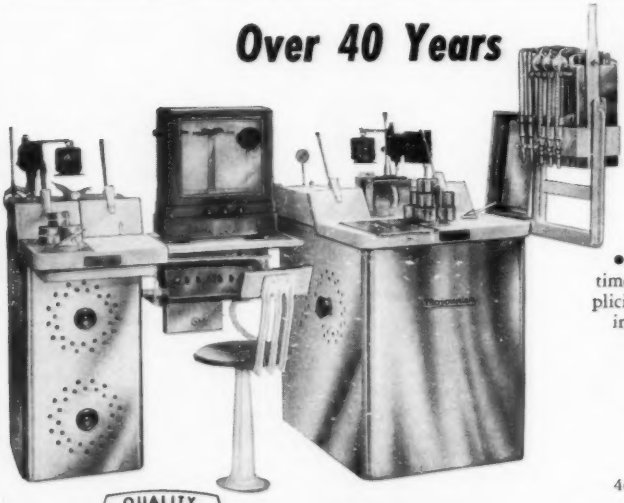

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Dairy Tech Club of Ohio State University

At the May 8 meeting of the Dairy Technology Club, officers were elected to serve for the coming year. The new president is Paul Culler. Paul, who will be a junior next year, was also in charge of preparing the scrapbook for display at the 53rd annual convention of the American Dairy Science Association.

Other officers are Roger Schelt, a senior, vice-president; Ron Klausing, a sophomore, treasurer; and Paul McClish, a junior, secretary.

Another interesting feature at the May meeting was the presence of two distinguished guests from India, Dr. Sersivet from Delhi and Dr. Sriekrishne from Madras. Drs. Sersivet and Sriekrishne are currently touring American colleges, studying American methods of procuring and processing milk and dairy products.

Dairy Science and Dairy Technology students planned a joint picnic for early in the month of June, climaxing a thrilling year of hard work.

Minnesota Dairy Science Club News

Mr. W. S. Moscrip who, in 1923, was chairman of the committee to formulate the first true-type model Holstein cow, was guest speaker at the club's January meeting. He reported on the development of the true-type

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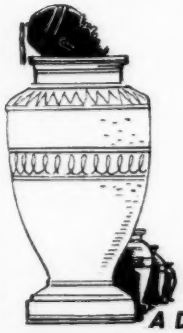

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models and on his experiences as an official classifier of dairy cattle.

Guest speaker at the February meeting was W. A. Gordon, Editor of the *Dairy Record*. His topic was "The Dairy Industry Today".

Three speakers highlighted the club's March meeting. Lew Conlon, manager of the Minnesota Dairy Industry Committee, reviewed the progress his organization has made in promoting dairy products. He then introduced Miss Judy Merritt, Minnesota's "Princess Kay of the Milky Way." Following Miss Merritt's appearance, H. W. Filk, President of the Minnesota Holstein Breeders Association, gave the club an interesting bit of his "milk stool philosophy."

Club activities during the winter quarter included sponsoring a booth at the Fund Fair, held to raise money for the new Student Union, and cosponsorship with the Union of a free milk hour. Over 15 gal. of milk, along with crackers and cheese, were served by Princess Kay. During two days of Farm and Home Week, the club served 268 dairy lunches. Proceeds from this event are used to finance club projects.

Officers for the coming year were elected at the March meeting. They are Donald Otterness, president; Warren Krause, vice-president; Harlan Stoehr, secretary, and Gerald Strandlund, treasurer.

Texas A & M Kream and Kow Klub Changes Name

At the January meeting, the members voted to adopt the name of Texas A and M Dairy Science Club.

During the past year, a total of $1,200 in awards on scholastic basis were received by six members of the Club. James Traweek, senior dairy major and president of the Club, received a $300 Borden Agricultural Award and a $100 Heep Award. Don Ancelin, Hagen Lippke, and Paul Payne each received $200 Heep Awards. Others receiving $100 Heep Awards were Frederick Gifford and Joel Gambrell.

Major activities of the Club during the year included a Christmas cheese sale, sponsoring a short course luncheon, and the annual Spring Dairy Show.

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PLANTS POISONOUS TO LIVESTOCK. A REVIEW

JOHN M. KINGSBURY
Department of Botany, Cornell University, Ithaca, New York

This paper deals with poisonous plants in relation to livestock, and emphasizes the present status of our knowledge concerning situations as they occur in veterinary practice in the United States. There are many poisonous principles found in or on plants which are potentially disastrous to livestock, but which have not yet been troublesome because of certain deep-seated protective feeding habits. With few exceptions, animals will not become poisoned by plants unless forced to do so by some unusual or artificial condition of husbandry. The important corollary of this statement, which can not be adequately developed in this paper, is that control of plant poisonings of stock is to be sought not in removal of the plant—an often costly and always temporary expedient—but by change in management.

Minimum standards for the successful elucidation of a problem involving one or more poisonous plants include: Description of management conditions which have led to poisonings, the kind and age of livestock affected, a competent veterinary description of symptoms and lesions, a competent botanical determination of the plant, proof that the plant identified is the etiological factor, both by demonstration that it has been eaten in sufficient quantity and by experimental creation of the symptoms, an experimental determination of the lethal dose in terms of percentage of the animal's weight or other basis, extraction and identification of the poisonous principle to kind at least (i.e., alkaloid, glycoside, etc.) and, finally, a preliminary investigation to determine if there is variation in toxicity of the plant with geographic location or with stage of growth. These requirements are rarely met, because it is uncommon to have the necessary botanical, chemical, and veterinary skills available in a given situation.

Muenscher *(158)* in his text treats 440 species of domestic poisonous plants. Some of these are excluded from the present discussion because they are solely dematitis-producing in human beings. On the other hand, a number of new species which have been shown to be toxic to stock since 1951 must be added. Far less than half of the total number of these species conform to the standards listed above. Of those plants reported, categorization can most easily be accomplished on the chemical nature of the toxic principles. These principles include alkaloids, glyc es (glucosides), including cyanogenetic and saponic, irritant oils, organic ac s, minerals including nitrates, selenium and molybdenum, resins or resinoids, phytotoxins, and toxic principles causing primary and hepatogenic photosensitivity. To these must be added a miscellaneous group of plants whose action is mechanical or whose toxic principle is unique or as yet partially uncharacterized. A few plants contain two or more toxic principles which are not in the same chemical group. In many cases, the chemical definitions of these groups are not on parallel grounds, and characteristics which identify a given moiety as a member of one may not exclude its concurrent

membership in another. Couch *(54)* has treated a number of these groups. Here it is intended briefly to characterize each toxic chemical moiety with emphasis on characteristics important in considerations of poisonous plants. A number of plants are listed for each category of toxic principle. The intention has been to include in these lists plants which reliably have been shown to have caused stock loss or sickness in the United States. It is difficult to draw a line between reliable and doubtful circumstantial reports.

Plants additional to those in this paper may be found listed as toxic in various compilations of poisonous plants. Sources of information for these additional reports are various; two predominate. Plants not domestically reported as poisonous may have been determined toxic to stock in other countries. Because of differences in management practices and growing conditions, it is unwise to use such information for other than casting suspicion on a given plant. For various reasons, it is similarly unwise to apply to livestock information gained solely from studies in human toxicology.

References in this paper were chosen on the basis of value, timeliness, and, to a degree, general availability.

In some instances, plants have been placed in a category on the basis of information developed from foreign studies which is not apparent in the domestic reference listed. On the other hand, mere chemical extraction of an alkaloid, glycoside, or other such compound from a toxic plant does not serve conclusively to incriminate that substance as the actual source of toxicity.

Alkaloids. Historically, alkaloids (*lit.* "alkali-like") are those products of chemical plant analysis which are not true bases (alkalis) but share certain chemical similarities with them. They are basic in reaction and form salts with acids. Generally insoluble in water but extractable in organic solvents, they occur as crystals (a few as liquids) in pure form, and in plants they are most often found as a soluble organic acid-alkaloid salt. They are almost universally bitter in taste. Alkaloids are found in less than 10% of all plant species, yet are common in certain families of plants. Further, alkaloids of similar structure are found in closely related plants, but on occasion identical alkaloids have been found in plants of no immediate relationship.

Most alkaloids exert a strong to very strong physiological reaction when introduced into an animal; a few produce no reaction. Activity is effected primarily via the nervous system by a mechanism at best poorly understood. The reaction may be quite specific for a given alkaloid in a given organism, yet may vary considerably both with different alkaloids in the same animal or with the same alkaloid in different animals. The major research interest in alkaloids is engendered by their importance in medicine and in human toxicology. Hundreds have been extracted and the molecular structure of many has been elucidated. Some have been synthesized. They are seen to be a heterogeneous assemblage of complex basic compounds containing nitrogen in heterocyclic and/or aromatic ring structure. A few similar substances of animal origin are often excluded by definition. Compounds answering the above description which may be found as normal intermediaries in metabolic pathways of plants and animals must be excluded.

The alkaloid content of a plant varies little, if at all, with factors such as nature of the growing season, climate, and availability of water. When present in a plant, alkaloids are frequently distributed throughout its structures. Any part may be dangerous to stock. Alkaloids may vary considerably with variety in cultivated plants. Their role in the plant has been variously ascribed. A view commonly held *(118)* is that they represent not particularly selective evolutionary "eddies" in plant nitrogen metabolism.

Some plant amines have physiologically toxic activity in animals. In this country, *Phoradendron* sp. (probably *Phoradendron villosum*) has been reported *(224)* toxic to cattle. The related *Phoradendron flavescens* contains phenylethylamine, which is credited *(42)* with a toxic action.

The genus *Astragalus* is large and complex. Some members are selenium accumulators *(q.v.)*. Others, together with species of the genus *Oxytropus*, are responsible for the nervous disease of horses, sheep, and cattle called "loco" *(128, 137)*. This syndrome has long been known in the West, where it has caused serious stock loss, and has been vigorously investigated; yet, it is still enigmatic in many ways. The syndrome itself is partially dependent in expression on the particular species of loco ingested. The poisonous principle has been stated *(81)* to be of alkaloidal nature. Recent work *(71, 82)* leaves the exact identity of the toxic compounds in doubt.

TABLE 1

Plants containing alkaloids toxic to stock[a]

			(References)
Aconitum sp.	Aconite, monkshood	Ranunculaceae	*7, 83*
Allium cepa	Cultivated onion	Liliaceae	*112*
Amianthium muscaetoxicum	Stagger grass	Lilaceae	*131*
Amanita muscaria	Fly agaric	(Basidiomycete fungus)	*28*
Argemone mexicana	Prickly poppy	Papaveraceae	*179*
Buxus sp.	Box	Buxaceae	*172, 217*
Claviceps sp.	Ergot	(Ascomycete fungus)	*59, 185*
Conium maculatum	Poison hemlock	Umbelliferae	*2, 55*
Corydalis sp.	Fitweed	Fumariaceae	*77*
Crotalaria sp.	Crotalaria	Leguminosae	*68, 168, 183*
Delphinium sp.	Larkspur	Ranunculaceae	*129, 130*
Dicentra sp.	Bleeding heart, etc.	Fumariaceae	*13, 64*
Festuca elatior	Fescue	Gramineae	*106, 116*
Gelsemium sempervirens	Jessamine	Loganiaceae	*217*
Lupinus sp.	Lupine	Leguminosae	*51, 87*
Peganum harmala	African rue	Zygophyllaceae	*14*
Senecio sp.	Ragworts, groundsels	Compositae	*138, 209, 210*
Sophora sp.	Mescal bean, sophora	Leguminosae	*15, 196*
Taxus sp.	Yew, ground hemlock	Taxaceae	*24*
Veratrum sp.	False hellebore	Liliaceae	*88*
Zygadenus sp.	Death camas	Liliaceae	*122, 123, 133*

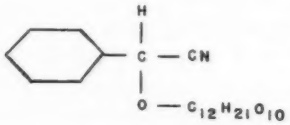
[a] For plants of the Solanaceae containing alkaloids toxic to stock, see text.

Glycosides. Glycosides are compounds which yield one or more sugars and one or more other compounds (aglycones) when hydrolyzed either by dilute mineral acids or by enzymes. The term glucoside has often been used synonymously with glycoside, and this practice continues *(154)*. Critical usage reserves the term glucoside for that particular kind of glycoside in which the sugar

component is glucose. Purified glycosides are usually bitter, colorless, crystalline solids. Glycosides are much more widely distributed in the plant kingdom than are alkaloids. Many are nontoxic (*e.g.*, several of the common nonphotosynthetic plant pigments). Both the sugar and aglycone components of a glycoside may be of a wide variety of compounds. Thus, the glycosides, too, constitute a grouping of heterogeneity.

Two subgroupings of glycosides, cyanogenetic and saponic, deserve particular attention.

Cyanogenetic glycosides. Glycosides which yield hydrocyanic acid (HCN) upon hydrolysis are termed cyanogenetic or cyanophoric (cyanogenic, the word sometimes used, is etymologically incorrect). A representation of the glucoside amygdalin, probably the most common cyanogenetic glycoside, is given here *(114)*.



It is found in many Rosaceae. The intact glycoside is not toxic *(53)*. The violent toxicity of the compound is caused solely by its HCN component, acting as a free molecule after hydrolysis. Little free HCN is found in healthy, actively growing plants. In natural circumstances hydrolysis is brought about by enzymatic action in the plant *(53)* or animal *(154)*. The frequently observed higher content of free HCN in wilted, frosted, or stunted plants may be caused by the joining of plant enzyme and cyanogenetic glycoside under these conditions, with resulting release of free HCN. Ruminant animals are held more susceptible to HCN poisoning from plants for the reason that the microflora of the rumen provokes greater enzymatic breakdown than that accomplished in nonruminants.

HCN is a small molecule readily taken into the blood stream and readily excreted by several routes. Chronic HCN poisoning as it is known in human beings is rare in animals. Not only are total cyanogenetic glycoside and free HCN fraction in the plant important in determining poisonings, but rate of ingestion and size and kind of animal also are important. There is little difference between toxic and lethal blood HCN levels. Two milligrams HCN per pound of animal per hour is close to the minimum lethal dose, and as a rule of thumb, plants which contain more than 20 mg. of HCN per 100 g. can be considered dangerous *(154)*. The well-known picrate test for cyanide has recently been adapted for easy field use on plant or stomach-content samples by Burnside *(29)*. Cases of poisoning are frequent under circumstances which allow animals unaccustomed access to dangerous pasture (such as immature sorghum) or browse (such as wilted wild cherry trimmings).

In the animal, HCN acts by inhibiting the action of the porphyrin enzyme cytochrome oxidase. This enzyme's function is to pass oxygen into the metabolic respiratory pathway. Thus, HCN poisoning constitutes asphyxiation at the cellular level. The ability of the blood to carry oxygen is unimpaired (of diag-

nostic importance). Death usually follows ingestion of a lethal dose within 15 min. to a few hours. Experimentally, it has been possible *(26)* to protect an animal from up to three times the minimum lethal dose by prompt injection of sodium thiosulfate and sodium nitrite, but the veterinarian usually can not arrive in time. This treatment (sodium thiosulfate) is designed to cause formation of the relatively harmless sulphocyanides and (sodium nitrite) to convert some of the blood hemoglobin to methemoglobin. Cyanide in the blood combines preferentially with methemoglobin to form the nontoxic cyanmethemoglobin *(84)*. The amount of nitrite injected is critical since sufficient unaltered hemoglobin must remain to carry a necessary minimum amount of oxygen to the tissue; otherwise nitrite poisoning will result (see later). Recent experiments with mice *(159)* have shown hydroxo-cobalamin (B_{12a}) efficacious in cyanide poisonings. Large amounts of this molecule, which combines preferentially with the cyanide radicle, safely may be used.

The content of cyanogenetic glycoside in a given wild plant or crop may vary widely with a number of external conditions. This variation has been explored most thoroughly in the case of sorghum *(80)*. It has been shown that factors such as climate, season, amount of rainfall, fertilization, and stage of growth are influencing. Heritable strain differences are important in sorghum. Hay made from some plants may be dangerous when cut but may become safe in time, possibly through volatilization of its HCN content.

TABLE 2

Plants containing cyanogenetic glycosides toxic to stock

			(References)
Acacia sp.	Acacia	Leguminosae	*1*
Bahia oppositifolia	Bahia	Compositae	*57*
Cercocarpus sp.	Mountain mahogany	Rosaceae	*1*
Hydrangea sp.	Hydrangea	Saxifragaceae	*217*
Linum usitatissimum[a]	Flaxseed, linseed	Linaceae	*104*
Phaseolus lunatus	Lima bean foliage	Leguminosae	*172*
Prunus sp.	Wild cherries	Rosaceae	*75, 99, 172*
Sorghum halepense[b]	Johnson grass	Gramineae	*136, 211, 225*
Sorghum vulgare[b, c]	Sorghum, Sudan grass, etc.	Gramineae	*22, 80, 211*
Suckleya suckleyana	Poison suckleya	Chenopodiaceae	*205*
Triglochin sp.	Arrow grass	Juncaginaceae	*48*

[a] See also Table 4.
[b] See also Table 7.
[c] See also Table 11.

Saponic glycosides. Saponins are large molecules which form a colloidal solution in water and produce a soapy froth or foam when agitated. They occur as amorphous glycosides (in which the aglycone is sometimes termed a sapogenin) and are of wide distribution in the plant kingdom. Many are nontoxic; those few of considerable toxicity have been termed *(5)* sapotoxins. In these the sapogenin is chemically related to the sterol hormones. The primary action of a saponin is to bring about a lysis of erythrocytes. It has been suggested *(5)* that this hemolytic action is due to combination of saponin with cholesterol in the membrane of the erythrocyte. Apparently, saponins are not readily absorbed

into the blood stream through the uninjured gut. Therefore, potentially hemolytic saponins are not toxic unless they also possess an irritant action sufficient to allow them to be absorbed into the blood stream through an injured gut wall. The major symptoms observed in cases of saponin poisoning are those of gastric irritation.

TABLE 3

Plants containing saponic poisonous principles

			(References)
Agrostemma githago	Corn cockle	Caryophyllaceae	*97*
Aleurites sp.	Tung tree	Euphorbiaceae	*67, 178*
Daubentonia drummondii[a]		Leguminosae	*121*
Daubentonia punicea[a]		Leguminosae	*217*
Glottidium vesicarium[a]		Leguminosae	*17, 72, 186*

[a] These plants are variously called coffeeweed, coffeebean, bagpod, rattlebrush, rattlebox, sesbane, and poison bean. Their Latin binomials are also confused, with *Sesbania* sometimes used as a collective generic name. There appear to be three entities that have been demonstrated poisonous. The binomials used here follow the treatment of Small *(191)*.

Other glycosides. Several plants have toxic principles which have been identified only as glycosides or as particular glycosides which are neither cyanogenetic nor saponic. Coumarin is a lactone, but probably occurs in plants in glycosidic combination *(153)*. Plants containing it which have proven toxic to animals under natural circumstances are the sweet clovers *Melilotus alba* and *Melilotus officinalis*. Coumarin is released from its glycosidic linkage and is changed to dicoumarin ($C_{19}H_{12}O_6$), a hemorrhagic agent, upon spoilage of sweet clover hay. Both natural and synthetic dicoumarin reduce blood prothrombin level in a similar fashion and cause the blood to be incapable of clotting *(200)*. Poisoned animals (usually cattle) will produce large subdermal weals in areas that become bruised, particularly about the neck and shoulders in stanchioned cattle.

The common foxglove *(Digitalis purpurea)* contains a number of so-called cardiac glycosides. Although this plant is available to animals in many parts of this country, cases of poisoning are absent or infrequent. Oleander *(Nerium oleander)*, which has been responsible for cases of poisoning in the southern United States *(227)*, contains a glycoside of similar action.

Irritant oils. Some plants contain fixed or essential (volatile-in-steam) oils which produce irritant properties of themselves or perhaps in some cases by

TABLE 4

Plants containing other glycosidic poisonous principles

			(References)
Aesculus sp.	Buckeye, horse-chestnut	Liliaceae	*32, 172*
Convallaria majalis	Lily-of-the-valley	Liliaceae	*88*
Helenium sp.	Sneezeweeds	Compositae	*126*
Ligustrum vulgare	Privet	Oleaceae	*172*
Linum neomexicanum[a]	Yellow pine flax	Linaceae	*65*
Melilotus sp.	Sweet clovers	Leguminosae	*200*
Nerium oleander	Oleander	Apocynaceae	*227*
Robinia pseudo-acacia[b]	Black locust	Leguminosae	*91*

[a] See also Table 2.
[b] Also stated to contain a phytotoxin *(84)*.

TABLE 5
Plants containing irritant oils

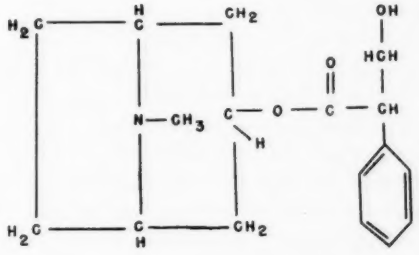
			(References)
Anagalis arvensis	Scarlet pimpernel	Primulaceae	*172*
Brassica sp.[a, b]	Mustards	Cruciferae	*90, 204*
Ranunculus sp.	Buttercups	Ranunculaceae	*155*
Thlaspi arvense	Fan weed	Cruciferae	*93*

[a] And other Cruciferae.
[b] See also Table 11.

reason of substances dissolved in them. In some, at least, they occur in glycosidic combination *(218)*. In general, in this country they are produced by a variety of plants that may cause low-grade poisonings if ingested in sufficient amounts. The irritant effect is produced in the gut and various degrees of gastroenteritis up to death will be produced, depending upon toxicity and dosage.

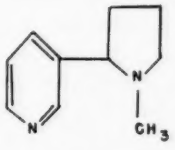
Solanaceous alkaloids. Special attention to this group of complex alkaloids, several of which are helpful in medicine, can evoke a number of useful conclusions. Solanaceous alkaloids may be divided into three major groups on the basis of chemical structure and physiologic activity. At least one plant known to have caused stock loss in this country occurs in each group.

The major alkaloid in the atropine group is hyoscyamine (isomeric with atropine) *(153)*.

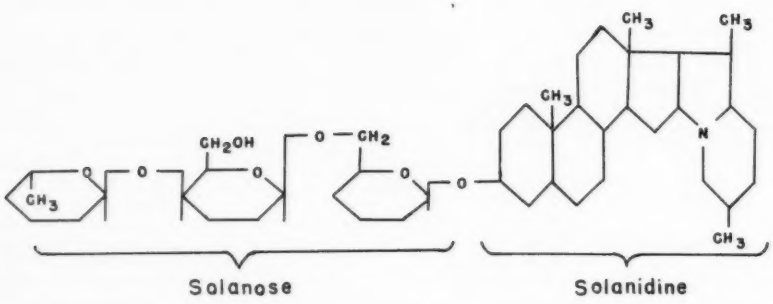


Plants containing this alkaloid, which have been poisonous to stock, include belladonna *(Atropa belladonna) (195)* and Jimson weed *(Datura stramonium) (37)*.

The major alkaloid in the nicotine group is nicotine *(118)*. Both wild *(134)* and cultivated *(172)* tobaccos (*Nicotiana* sp.) have caused stock loss.



Species of nightshade *(Solanum* sp.*)* and some others contain solanine as their major alkaloid *(119)*.



Plants whose toxic principle falls in this group include potato *(115)*, tomato *(37)*, and several wild species of the genus *Solanum (34, 172, 217)*.

These formulas are figured so that several conclusions may be drawn: *(a)* Alkaloids within a group of closely related plants (such as Solanaceae) may chemically be very dissimilar. *(b)* On the other hand, a given alkaloid may be found in a wide variety of plants. For instance, nicotine is found not only in various tobaccos but also in a wide variety of plants including two unrelated genera *(Lycopodium* and *Equisetum)* of the spore-bearing division of the plant kingdom *(118)*. *(c)* On the other hand, an alkaloid characteristic of a given plant species may be found in a number of other species closely related to it. For example, well over a dozen species of *Solanum* contain solanine or a molecule closely similar to it. *(d)* A toxic principle may fall into more than one category on the basis of its structure and physical properties. For example, solanine is an alkaloid by reason of its nitrogenous nature and basic reactions. It is also a glycoside, since chemically it consists of a sugar (solanose) linked to a nonsugar or aglycone moiety (solanidine). Since it is a molecule which forms a stable foam when shaken with water, additionally it answers the definition of a saponin. Nicotine is one of the very few alkaloids which also has the physical characteristics of a volatile oil.

The Solanaceae can be used to demonstrate the relative uselessness of common names *(192)* and the necessity of a botanical determination of the plant in question. To choose two solanaceous plants as illustrative, we find the following confusing items in the literature: *Atropa belladonna*, whose active principle is of the atropine group, is commonly called belladonna *(158)*, but also deadly nightshade *(41, 195)*. *Solanum nigrum*, whose active principle is of the solanine group, is commonly called black nightshade *(158)*, but also deadly nightshade *(37, 101)*. This confusion in common names leads to confusion in plants and poisonings. Again from the literature, deadly nightshade is equated with belladonna without botanical identification of the plant in question *(12)*. Deadly nightshade, identified as *Solanum nigrum*, causes belladonna poisoning *(101)*, with symptoms including widely dilated pupils (characteristic of the atropine group, but either unexpressed, or expressed very mildly, in poisonings by plants whose major poisoning principle is of the solanine group). Deadly nightshade is identified as *Solanum nigrum*, but black nightshade is botanically unidentified and gives

a strong cat's-eye test for atropine *(37)*. All poisonous Solanaceae produce belladonna poisoning *(101)*. Members of the Solanaceae are collectively termed nightshades. Many, perhaps the majority, are potentially lethal. These examples show that the term deadly nightshade is a poor identification for a solanaceous plant.

Organic acids. Oxalic acid is toxic not because of its acidity but because of the specific action of the oxalate ion. It is the only organic acid in plants that is toxic to stock under natural conditions. It occurs in plants in the form of soluble (sodium and potassium) and insoluble (calcium) oxalates. Insoluble oxalates pass through the animal gut without being absorbed into the blood stream and are, therefore, nontoxic. Many plants contain small amounts of soluble oxalates; only a few contain enough to be toxic. A maximum is found in *Halogeton* (a recent Russian immigrant) which may contain as much as 28% soluble oxalates. The content of soluble oxalates in plants varies widely with season, reaching a maximum in late summer and fall. The Utah Experiment Station has recently investigated *Halogeton (49)* and oxalic acid poisoning. Soluble oxalates are readily eliminated from the blood by the kidneys. Both the amount of oxalate consumed and the time required for its consumption are important factors in determining when the toxic level of blood oxalate will be reached. Roughly one ounce of soluble oxalate is lethal to a sheep. Presence of food in the stomach reduces the rate of absorption. About half again as much is required for symptoms to be developed under these circumstances. A total of several times these amounts may be ingested without untoward effect if taken in small amounts over the period of a day.

Ingestion of a toxic dose of soluble oxalates is followed in a few hours by death after general symptoms of dullness, depression, and a final comatose state. Incoordination and stiffness, especially of extremities, are commonly observed. The uptake of soluble oxalates into the blood stream from the gut results in the precipitation of calcium oxalate from the blood serum. Calcium ion content of the blood will be lowered, yet total calcium may change but little, especially at first. The immediate cause of death is difficult of determination, but appears to be the result of asphyxiation or heart failure brought on by acute hypocalcemia. In addition, crystals of calcium oxalate will be formed in the kidney tubules, especially of the cortex, sometimes in such great quantity that practically every tubule is filled. Rupture of the tubules and other degenerative changes in the kidney follow.

Experiments at Utah have shown that injections of calcium gluconate help little if at all in cases of poisoning, but feeding of calcium-rich feed along with

TABLE 6

Plants that may contain toxic concentrations of oxalic acid

			(References)
Halogeton glomeratus	Halogeton	Chenopodiaceae	*49*
Oxalis sp.	Sorrels	Oxalidaceae	*35*
Rheum rhaponticum	Rhubarb	Polygonaceae	*172*
Sarcobatus vermiculatus	Greasewood	Chenopodiaceae	*228*

FIG. 1. Section of kidney showing oxalate crystals. Prepared at Utah State Agricultural College from sheep killed experimentally by *Halogeton* poisoning.

the dangerous plants may be beneficial. The greater the amount of soluble oxalate precipitated in the gut, the less will be available to produce a toxic reaction.

Mineral poisonings. Plants may be made toxic secondarily through deposition on their surfaces of certain minerals such as arsenic or fluorine *(98)* in the vicinity of particular industrial installations. Such situations are usually obvious and are not detailed here.

Nitrate. Poisoning by nitrate or compounds elaborated from it has been known in this country since the late 19th Century but has recently assumed new dimensions. Many crop plants (especially, oat hay, corn, and sorghum), vegetables and weeds (especially of the amaranth, goosefoot, mustard, composite, and nightshade families) have been found on analysis to contain nitrate (usually as the potassium salt) at a level potentially toxic to animals. Several have been definitely incriminated in cases of poisoning. Plants are considered potentially dangerous when they contain more than 1.5% nitrate (as KNO_3, dry weight) *(18)*. Recently *(40)*, subclinical nitrate poisoning has been attributed to a feed intake nitrate level of 0.5%.

Nitrate poisoning has recently been reviewed by Whitehead and Moxon *(221)*. It can be produced by ingestion of nitrate fertilizers, machine oil, natural well and pond water of high nitrate or nitrite content, pickling brine and the like, as well as by plants and hay or silage made from them. Nitrite formed from nitrate in the animal gut is about ten times as toxic. Reduction appears to be effected more quickly and easily in the ruminant digestive process when compared with the nonruminant. Nitrite impairs the blood's ability to transport oxygen. Ferrous hemoglobin picks up oxygen in a chemically loose combination, releasing it easily to the tissues on demand. Nitrite causes oxidation of ferrous hemoglobin to ferric hemoglobin, termed methemoglobin, which can not transport oxygen. This compound causes the blood to become dark chocolate–brown in color (diagnostic in nitrite poisoning). Death through asphyxiation (anoxia) occurs when about three-fourths of the blood hemoglobin is converted to methemoglobin and usually eventuates within a few hours to a day after ingestion of a lethal dose. Effective treatments may be attained by injection of methylene blue *(19)*. This reducing agent causes ferric methemoglobin to change to ferrous hemoglobin, which again can carry oxygen. In pregnant animals the fetus is particularly sensitive to a reduction in oxygen in the parent's circulation. In mild cases of nitrate poisoning, abortion may result *(38)*.

Additional kinds of poisonings by various combinations of nitrogen have recently come to light. Nitrite (NO_2) can be reduced still further to a nitroso (NO) radical. Nitrosohemoglobin exhibits many of the color and toxicity characteristics of carbon monoxide hemoglobin. Poisoning via the formation of nitrosohemoglobin in the blood may occur under natural conditions *(40)* and is distinguishable from carbon monoxide posoning by obvious differences in the source of the poisonous principle or by a simple test as detailed by Case *(40)*. Abnormally high concentrations of nitrate in forage being ensilated lead to dangerous and even explosive fermentations in the silo. Decomposition of nitrate in an acid medium results in the production of nitrogen dioxide, a toxic, yellow-brown gas. This has been responsible for toxicity in human beings (silo-filler's disease) *(58)* and recently has been shown to be toxic to cattle *(182)*. In the latter situation, nitrogen dioxide may have been generated directly in the bovine rumen. Treatment with intrasmuscular injection of adrenocortical steroids has been suggested in animal practice *(3)*. Although silage fermentation results in a decreased nitrate content, the silage may retain enough nitrate to be toxic for a considerable time.

A number of factors may influence the amount of nitrate contained in plants. Soil nitrogen content, both original and after fertilization, amount and kind of other elements in the soil, amount of light, and availability of water during the growing period, the kind of plant, and the stage of its growth are all important. Treatment with the herbicide, 2,4-D, has been shown to produce two unexpected results *(199)*: First, a number of normally unpalatable weeds, such as pigweeds (*Amaranthus* sp.), ragweeds (*Ambrosia* sp.), and Jimson weed *(Datura stramonium)* are made palatable. Second, the resulting upset in nitrogen metabolism may cause the treated plant to accumulate nitrate to a toxic level. A convenient field test for nitrate and nitrite is given by Case *(40)*.

TABLE 7

Some plants that may contain toxic concentrations of nitrate

			(References)
Weeds:			
Amaranthus sp.	Pigweeds	Amaranthaceae	
Carduus sp.	Bull thistle	Compositae	
Chenopodium sp.	Pigweed, lamb's quarters	Chenopodiaceae	
Cirsium arvense	Canada thistle	Compositae	
Convolvulus sp.	Bindweeds	Convolvulaceae	
Franseria discolor	White ragweed	Compositae	
Helianthus annuus	Wild sunflower	Compositae	
Kochia scoparia	Fireball	Chenopodiaceae	
Lygodesmia juncea	Skeleton weed	Compositae	
Panicum capillare	Witchgrass	Gramineae	*18, 86, 221*
Rumex sp.	Dock	Polygonaceae	
Salsola pestifer (= S. kali)	Russian thistle	Chenopodiaceae	
Solanum sp.[a]	Nightshades	Solanaceae	
Sonchus asper	Sow thistle	Compositae	
Crop plants:			
Avena sativa[b]	Oat hay	Gramineae	
Hordeum vulgare[c]	Barley	Gramineae	
Sorghum vulgare[b, d]	Sudan grass	Gramineae	
Triticum aestivum[c]	Wheat	Gramineae	
Weeds:			
Amsinkia sp.[c]	Tarweeds	Boraginaceae	*11*
———	Bluegreen algae[b, c]	(Cyanophyta)	*40*
Datura sp.[a]	Jimsonweed	Solanaceae	*40*
(Echinochloa crusgalli)	Barnyard grass	Gramineae	*40*
Euphorbia maculata[b]	Milk purslane	Euphorbiaceae	*40*
(Polygonum)	Smartweeds	Polygonaceae	*40*
Silybum marianum	Variegated thistle	Compositae	*109*
(Sorghum halepense)[d]	Johnsongrass	Gramineae	*40*
(Stellaria media)	Chickweed	Caryophyllaceae	*40*
Thelypodium lasiophyllum	Mustard	Cruciferae	*11*
Crop plants:			
Beta vulgaris	Beet and mangold	Chenopodiaceae	*180*
(Ipomoea batatas)	Sweet potato vines	Convolvulaceae	*20*
(Medicago sativa)[b]	Alfalfa	Leguminosae	*40*
Zea mays[c]	Corn	Gramineae	*66, 220*

( ) Indicates reference cited did not give scientific name identification.
[a] See also Solanaceous Alkaloids, in text.
[b] See also Table 11.
[c] See also Table 13.
[d] See also Table 2.
[e] And other Cruciferae.

Selenium. Of plants which vary in toxicity with geological formation, those which take up selenium are by far the most important. Stock loss, now known to have been caused by ingestion of selenium, has been reported from as early as 1857, but sustained work to elucidate the causes dates only to 1932. Diseases locally called with various degrees of inappropriateness blind staggers and alkali disease as well as an acute poisoning syndrome, have since been shown to be caused by selenium ingestion. Major contributions to the study of the geology, geography, and biological implications of naturally occurring selenium deposits have come from the South Dakota and Wyoming Experiment Stations and from the late S. F. Trelease at Columbia University. Much has been learned, yet certain observations remain obscure and several gaps in information exist. The massive accumulation of data has been reviewed by Moxon and Rhian *(157)* of

the South Dakota group, and in a relatively nontechnical presentation by Trelease and Beath *(207)* (the latter of the Wyoming group).

The nonmetallic element selenium is found in various forms in soils and rocks of geological formations from Pennsylvanian to Recent, particularly the Cretaceous, in 15 western states. Because of the small amounts of total selenium which rarely exceed 100 p.p.m., determination of the form of selenium in the soil and rocks has been difficult. It appears that some amounts of elemental selenium, and certain selenides, selenites, and selenates are present in varying ratio. Elemental selenium and the selenides are relatively insoluble. Some selenites and selenates are insoluble, others are soluble. These forms are originally of geologic origin, but physical, chemical, and perhaps biological forces cause a degree of interconversion. Organic selenium compounds are also found in the soil but are strictly of biological origin. Some are soluble; some are not. Soil selenium concentration will vary from a trace to not more than 150 p.p.m., with the majority of soils containing less than 5 p.p.m. The fraction of total selenium that is soluble and, therefore, available for plant uptake, is usually less than 50%. Amount and kind of other soil constitutents, especially sulfur, also influence the availability of soil selenium to plants. These factors are more operative in the root-bearing soil horizon than at the soil surface.

Plants show a remarkable and unexpected relationship to selenium in the soil. They accumulate it or they do not. The strength of selenium accumulation exhibited by some plants is considerable. The accumulators may be further divided into obligate and facultative selenium species.

Obligate selenium species seem to require selenium for proper growth and will be found *only* where there is selenium in the soil. For this reason they are termed *indicator plants (8)* throughout the selenium literature and have been useful in pointing to selenium-bearing geologic formations of potential toxicity. These plants invariably contain selenium during the greater part of their life cycle and maximum tissue concentrations have been reported *(207)* approaching 15,000 p.p.m. Their selenium content, determined by chemical analysis, can be used as an index of toxicity for the soil bearing them. Twenty-four species and varieties of *Astragalus,* all species so far examined of *Xylorrhiza* (woody aster), all species examined of *Oonopsis* (goldenweed), and all species examined of *Stanleya* (prince's plume) have been found to be reliable selenium indicator plants. All of these are conspicuous elements in the areas where they occur. There are some 300 American species in the genus *Astragalus.* Those accumulating selenium fall into genetically related groups which can be recognized as such by criteria other than selenium accumulation. Four species, *A. racemosus, A. bisulcatus, A. pectinatus,* and *A. pattersoni,* are most useful as indicators by reason that they enjoy a wide geographic distribution. One or more is found in Montana, North and South Dakota, Nebraska, Kansas, Oklahoma, Texas, Montana, Wyoming, Colorado, New Mexico, Idaho, Utah, Arizona, and Nevada. With the exception of California, these are all the states in which selenium is found. *Astragalus preussii* is an indicator species which is found in California.

The facultative selenium species or, as commonly called, the *secondary*

selenium absorbers, are not limited to soils containing selenium. They will accumulate selenium when growing in seleniferous soils but, other conditions being equal, will grow just as well on soils containing no selenium. They are commonly the cause of stock poisoning, for among them are a number of genera (for example, *Aster*, *Atriplex*, *Sideranthus*, and *Machaeranthera*) that are grazed, and on nonseleniferous soil may be palatable and valuable forage plants. Fortunately, increasing selenium content tends to make a given species increasingly unpalatable.

Most plants are nonaccumulators. However, any plant may passively take up selenium dissolved in the soil. The amount taken up will be small and will be determined by the amount available in the soil. Moderate amounts of selenium exert a markedly toxic effect in many plants, which become stunted or are otherwise harmed. Within the genus *Astragalus*, some species (indicators) require, are stimulated by, and vigorously accumulate quantities of selenium. Others are inhibited in growth or killed by selenium and take it up only passively. Growing on the same seleniferous soil, the former species may contain several thousand parts per million, and the latter only a trace. The basis of the accumulatory power of obligate and facultative species is unknown. Some experimental results indicate that the accumulatory power is not to be correlated with any particular form of selenium in the soil; all kinds of available selenium are taken up in greater amount by an accumulator plant than by a nonaccumulator.

In summary, the amount of selenium in a given plant will be determined by its selenium-accumulating power and the soil's selenium-supplying power, The former is determined, in turn, by the species of plant, its stage of growth, and its general vigor. The latter is determined by the form of selenium, its concentration in the root zone, and the amount and kind of other elements present. As a rough approximation, if the amount of selenium in an indicator species on a given soil is 100 ×, then the amount in a facultative species will be of the order of magnitude of 10 ×, and in a nonaccumulating species, of ×. Five p.p.m. selenium renders a plant potentially toxic. The higher amounts of selenium in plants can be detected by the characteristic garlicky odor which they impart.

Of the nonaccumulators, both native range plants and crop plants may occasionally build up toxic levels of selenium. There are few natural soils containing a high enough level of soluble selenium to cause nonaccumulators growing on them to be potentially toxic. Such soils are found in limited areas of western South Dakota, northern Nebraska, northeast Wyoming, and in a few other places. More important is the toxicity conferred upon nonaccumulators, especially cereal plants, through the action of converter plants. As has been seen, obligate and facultative selenium plants can accumulate high concentrations of selenium. Selenium, concentrated thus, is returned to the soil upon leaf drop, by death of the accumulating plant, or through its being plowed under. The local concentration of soluble selenium compounds thus may be increased to a point at which toxicity may be imparted to those nonaccumulating plants subsequently growing there. Native grasses and other plants growing within a few feet of a perennial converter plant thus may be made toxic. Entire fields of grain or

crop plants may be made toxic if grown where a stand of converter plants was plowed under.

Animal disorders caused by ingestion of selenium are determined by the amount and form of the selenium ingested. Three basic syndromes are presented. Cattle, sheep, swine, and horses may be poisoned; the form, severity, and frequency are in part determined by the different habits of the animals.

When plants containing more than about 250 p.p.m. selenium are ingested, *acute selenium poisoning* is produced. Obligate selenium species, particularly of the genus *Astragalus*, are usually responsible. Poisoning has occasionally been severe under range conditions where these plants grow. Death in a few hours to days follows ingestion of a massive lethal dose. The pathology of this and the two following forms of selenium poisoning have been detailed by Rosenfeld *et al. (174)*. In acute poisoning much of the pathologic picture of necrosis and hemorrhage can be attributed to direct injury of capillaries by selenium.

A chronic selenium poisoning, rather inappropriately termed blind staggers, results from the continued ingestion of native-range plants containing small to moderate amounts of selenium (usually less than 200 p.p.m.). Whereas some time is required for the development of this disease, the onset of symptoms often is abrupt. Severe nutritional disturbances result in wasting. Variable impairment of vision to complete blindness in cattle correlates with decrease of vitamins A and C. A depraved appetite and terminal paralytic stage are typical. Death is the immediate result of respiratory failure. Injection of strychnine sulfate and drenching with warm water is an effective treatment if undertaken before the final paralytic stage.[1]

A chronic selenium poisoning, equally inappropriately termed "alkali disease", results from the continued ingestion of crop plants such as corn, wheat, barley, oats, grass, and hay containing 5 to 40 p.p.m. selenium. Emaciation, lameness, and loss of long hair are typical. The most characteristic feature is hoof deformity, which may become severe enough to result in sloughing of all four hooves. Death, which may occur after two months on seleniferous feed, is often the result of the refusal of the animal to move and is via starvation or thirst.

The difference in clinical syndrome between blind staggers and alkali disease may be correlated with the form of selenium in cereal crops (entirely organic, roughly half-soluble), as compared with that in range plants. Selenium content of hair may be used as a diagnostic aid in borderline cases of alkali disease *(163)*. In areas where grain crops regularly contain small amounts of selenium, addition of very small amounts of inorganic arsenic to the seleniferous ration has greatly lessened its toxic effect. However, there has been a tendency for arsenic to accumulate in animal tissues to an extent that might make meat dangerous for consumption. Recently, it has been shown *(213)* that various

[1] Recent investigations in Colorado *(107)* report a condition, locally called "forage poisoning," of identical clinical syndrome with "blind staggers." "Forage poisoning" occurs in feed lots and in pastured cattle and sheep. Neuropathologic changes are reported. Doubt is cast on selenium as the etiological agent.

TABLE 8

Plants which may accumulate selenium

			(References)
Obligate or indicator:			
Astragalus, certain species[a]	Poisonvetches	Leguminosae	
Stanleya, all species	Prince's plume	Cruciferae	
Oonopsis, all species[b]	Goldenweeds	Compositae	
Xylorrhiza, all species	Woody asters	Compositae	
Facultative or secondary selenium absorbers:			
Aster sp.[c]	Aster	Compositae	
Atriplex sp.	Saltbushes	Chenopodiaceae	*9, 10, 207*
Castilleja sp.	Paint brushes	Scrophulariaceae	
Commandra pallida	Bastard toadflax	Santalaceae	
Grayia sp.	Hop-sage	Chenopodiaceae	
Grindelia sp.	Gumweeds	Compositae	
Gutierrezia sp.	Snakeweed	Compositae	
Machaeranthera sp.[c, e]	Tansy aster	Compositae	
Penstemon sp.	Beard tongue	Scrophulariaceae	
Sideranthus sp.	Ironweed	Compositae	

Crops which have taken up toxic levels of selenium include corn, wheat, oats, barley, grass, and hay.

[a] Of the species groups *bisulcatus, diholcos, pectinatus,* and *grayi*, especially.

[b] Sometimes lumped with the genus *Aplopappus*. (See also Table 13, and footnote.)

[c] May be grazed.

[d] See also Table 13.

[e] Sometimes lumped with the genus *Aster*.

organic arsenicals are effective in counteracting the baleful influences of selenium in pigs; yet, they are lost from the tissues within five to ten days after cessation of treatment.

Molybdenum. Molybdenum is another mineral element occasionally found in soils in sufficient quantity to cause the vegetation growing on them to be toxic to stock. Molybdenum poisoning was first recognized in England (where it is called teartness), and has since been reported in this country *(23, 223)*, principally from the San Joaquin Valley in California. Soils containing more than 7 p.p.m. molybdenum are considered potentially dangerous. The element is accumulated in the animal body and symptoms will appear from one to seven months after dangerous forage is continuously grazed. Well-cured hay is less dangerous. Nonruminants are relatively resistant.

Prominent symptoms include emaciation, scours, and change in coat color. There is usually a severe anemia. It is possible that the action of molybdenum is effected by causing an inbalance in minerals, particularly copper, in the animal's nutrition. Treatment with soluble copper compounds is useful *(102)*.

Resins or resinoids. Active principles of considerable virulence found in some plants are classified chemically as resins or resinoids. These terms denote a grouping for convenience of a heterogeneous assemblage of complex compounds, differing widely in chemistry but united on the basis of certain shared physical characteristics. Upon extraction, they are solid or semisolid substances at room temperature, are brittle and easily melted or burned. They are soluble in a number of organic solvents but insoluble in water and do not contain nitrogen.

Physiologic activity is effected by direct irritation of nervous or muscle tissue and is sometimes quite specific. Plants of widely differing relationships

may contain resins, but a few plant families are noted for their content of the same or similar resins among their several species. Certain resin-bearing plants are among the most dramatic poisoners of human beings as well as animals. One such is *Cicuta maculata*, water hemlock, which produces major symptoms of violent convulsion.[2] This plant, widely distributed in the United States, is responsible for a human mortality probably second only to that caused by ingestion of poisonous mushrooms. It should not be confused with the closely related poison hemlock *(Conium maculatum)*, whose toxic action is alkaloidal. Both may cause stock losses. Neither should be confused with true hemlock *(Tsuga)*, an evergreen needle-bearing tree not known to be toxic to stock, or ground hemlock *(Taxus)*, whose toxic principle is an alkaloid.

TABLE 9

Plants containing resinoid poisonous principles

			(References)
Asclepias sp.[a]	Milkweeds	Asclepiadaceae	*54, 196, 208*
Cicuta sp.	Water hemlock, poison parsnip	Umbelliferae	*79, 105*
Iris sp.	Iris, flag	Iridaceae	*25*
Kalmia sp.[b]	Mountain laurels	Ericaceae	*46, 170*
Leucothoe sp.[b]	Laurels	Ericaceae	*63*

[a] Species of *Asclepias* vary greatly in toxicity. Some are nontoxic. There has been botanical confusion among the toxic ones *(196)*.

[b] Several other members of the Ericaceae (*Rhododendron, Pieris, Lyonia, Azalea,* and others) contain the same or similar resinoid *(54)*.

TABLE 10

Plants containing phytotoxin poisonous principles[a]

			(References)
Abrus precatorius	Precatory bean	Leguminosae	*216*
Ricinus communis	Castor bean	Euphorbiaceae	*226*

[a] See also *Robinia pseudo-acacia* (Table 4).

Phytotoxins. Phytotoxins (also called toxalbumins) are protein molecules of high toxicity produced in a small number of plants. Few native plants produce them and those that do are rarely the cause of livestock loss. Phytotoxins are large, complex molecules similar to bacterial toxins in structure and causing similar reactions in animals. Like bacterial toxins they act as antigens, eliciting an antibody response in the reacting animal. Toxicity is conferred by an ability to cause agglutination of erythrocytes. True immunity can be produced through administration of immunizing doses of increasing strength. Passive immunity can be conferred by injection of antisera. Antisera are commercially unavailable because of the rarity of cases. Phytotoxins are nondialyzable, heat-labile, and can be positively identified by precipitin reactions with sera containing known antibodies. Toxic castor-bean press cake can be made nontoxic simply by heating.

Photosensitization. Under certain circumstances animals become hypersensi-

[2] An intensely graphic, though unscientific, description of a poisoning by *Cicuta*, is that by J. Wepfer (1679), which is found translated from the original Latin as "Case VI" in Nevada *Tech. Bull.* 81 *(105)*.

tive to light. Compounds in certain plants may bring about hypersensitivity in a direct or indirect fashion. Certain compounds of various nonplant origins, both within and without the animal, may also effect hypersensitivity; these are not discussed in the following. Clare *(44)* has thoroughly reviewed the extensive literature on photosensitization. His terminology is used in the following summary, which intends to relate the kinds of photosensitization with the plants shown responsible for producing cases of photosensitization in this country.

The exact mechanism by which photosensitization is produced is not understood, but from other biological situations in which light effects changes in living systems, it appears that the basic reaction is an oxidation enhanced by energy from incident light. Light is trapped and made chemically available by a light-receiving substance. Although such substances usually absorb specific wave lengths of light in the visible region of the spectrum and, therefore, are pigments, a substance may absorb light in the ultraviolet region of the spectrum and be photodynamic even though colorless. The wave length(s) of absorption, and particularly the wave length(s) at which the engendered reaction is greatest, can be used to characterize the light-absorbing substance. Plant-caused photosensitivity thus can be distinguished from true sunburn by differences in activating wave lengths. In photosensitization, light-catalyzed reactions—the details of which are not clear—effect a greatly increased permeability of the capillary wall in the surface beds where reaction takes place. The presence of both a reactant substance in the peripheral circulation and of light are required. Therefore, the reaction will occur only in areas of unpigmented or lightly pigmented skin which are not covered by a dense light-screening coat of hair. In classical photosensitization, white sheep are affected about the head, and cattle on white or unpigmented areas of the skin, but particularly about the udder. Black sheep are resistant. However, Case *(36, 39)* presents conflicting evidence in which animals show photosensitizations in pigmented parts of the body. Pathology is remarkably similar no matter what the source or chemical identity of the reactant substance. In animals, restlessness is followed by a reddening of the skin (erythema), which in turn is superseded by an edematous infusion under the skin of affected parts, caused by leakage of serum from the capillary beds. In severe cases there will be a serous seepage through the skin. Necrosis follows and in time varying amounts of skin will slough off, depending upon the severity of the photosensitization. In seriously affected sheep the lips may be lost and ears may be seriously deformed. Secondary invasion of necrotic areas by bacterial infection is common. The photodynamic action, by itself, is not usually lethal, but death is common by reason of starvation, liver damage (see below), or other secondary effects. In sheep that recover, areas of affected skin will not grow further wool.

Photosensitivities may be divided into types on the basis of the nature of the photodynamic substance, and particularly its source. In photosensitizations caused by plants, the photodynamic substance may come directly and unchanged from the plant (Type I of Clare), or it may be a normal breakdown product in digestion usually eliminated by the liver (Type III of Clare). The former may

be called primary photosensitivity, and the latter may be termed hepatogenic photosensitivity. Sometimes the latter is termed ictrogenic photosensitivity, from the fact that a pronounced icterus throughout the animal is a common symptom.

Two plants occurring in the United States have been shown to produce primary photosensitivity. Both were known as photosensitizers in Europe and had been early investigated before cases were reported in this country. *Hypericum perforatum* (St.-John's-wort, or Klamath Weed) is widely distributed as a common weed, particularly in the Northeast and the Northwest. *Fagopyrum sagittatum* (buckwheat) is a crop grown for its grain. The vegetation from either plant contains a pigment (probably not the same) which passes the gut wall, enters the circulation, is not eliminated by liver or kidney and, therefore, reaches the peripheral circulation. All symptoms produced by either of these plants are solely those of photosensitization. In neither case has the pigment been conclusively identified, although attempts at its elucidation have been made. In the case of *Hypericum*, it has been incompletely identified *(165)* as a partly reduced polyhydroxy derivative of helianthrone. Three substances, experimentally shown photodynamic, have been isolated *(215)* from buckwheat, but have not been chemically identified. Cases produced by ingestion of *Hypericum* are not common, and those produced by ingestion of buckwheat vegetation are rare in this country. It is interesting to note that elimination of *Hypericum* has been the objective in one of the more publicized attempts at biological control (through use of insects of the genus *Chrysolina*).

Hepatogenous photosensitivity has been investigated most thoroughly in Africa. In this and other work it has been determined that the photosensitizing plants contain a toxic compound (not completely elucidated for any given case) which produces injury of the liver and liver dysfunction. Normally, the liver eliminates (in the bile) a number of usual breakdown products of digestion The injured liver in hepatogenous photosensitivity is unable to eliminate certain pigments found as normal breakdown products in digestion and which, therefore, may reach the peripheral circulation and cause photosensitization. It has been shown in more than one such plant that the porphyrin, phylloerythrin, a normal product of animal digestion of chlorophyll, is the photodynamic pigment and is caused to reach the peripheral circulation by dysfunction of the liver. It may which are absorbed through the gut and reach it through the portal circulation. prove to be the universal photodynamic pigment in this type of photosensitivity, or it may not. A method of testing for hematoporphyrin in the urine is given by Sippel and Burnside *(188)*.

Much of the African work has been with *Tribulus terrestris*, which produces bighead (Geeldikkop) in sheep. This plant (commonly called caltrop, or puncture vine) is found in several of the western states, but cases of photosensitization by it in this country appear to be rare. The plant which produces the heaviest domestic loss by photosensitization is the horse brush, *Tetradymia*, a common brushy plant of several of the western states. Typical bighead in sheep is produced within 16 to 24 hr. after ingestion of a toxic dose. It is accompanied by liver damage, but neither the liver toxin nor the photodynamic pigment has

been identified. If sufficient of the normally distasteful plant is ingested, death will follow within one day directly as a result of liver injury and without the development of photosensitization symptoms.

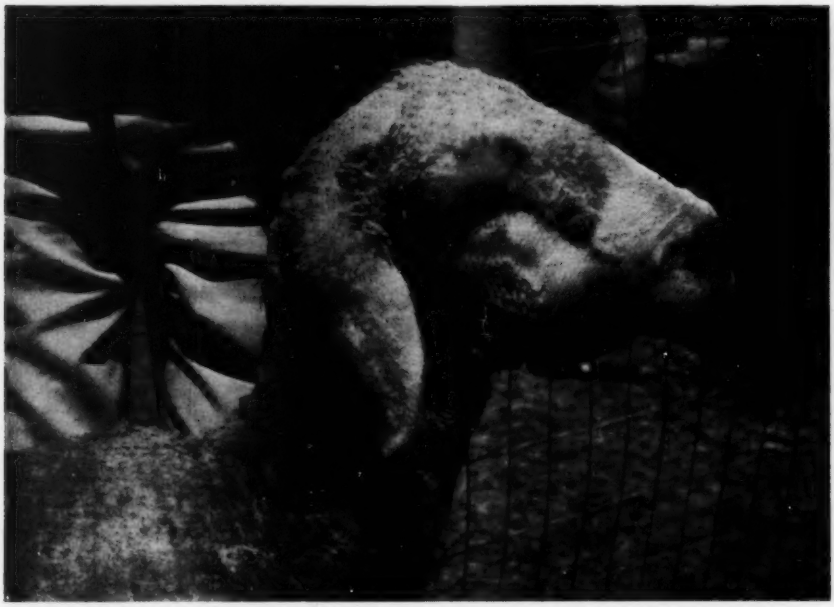


FIG. 2. Sheep A. Typical photosensitization ("bighead") in sheep produced experimentally at Utah State Agricultural College by feeding *Tetradymia*.

FIG. 3. Sheep B. Fed twice as much *Tetradymia* as Sheep A, Sheep B developed no symptoms of photosensitization, but quickly exhibited symptoms of, and died from, acute liver injury.

Agave lecheguilla, the lechuguilla of the Southwest, produces photosensitization accompanied by liver damage. Certain experimental results *(142, 143)* indicate that the liver damage may not be directly responsible for the photosensitization, but that the latter may be caused directly by a pigment contained in the plant. *Nolina texana*, sacahuiste, also of the Southwest, produces liver damage in sheep, goats, and cattle which under certain circumstances is also accompanied by photosensitization. The buds, blooms, and mature fruits are the parts of the plants which are normally ingested. These contain no chlorophyll. If the animal ingests no chlorophyll from other sources photosensitization does not follow, but it usually develops if the diet contains other green feed. Photosensitizations have also been reported in this country as being produced by ingestion of a water bloom *(21)*. The bluegreen alga, *Microcystis flos-aquae*, has been incriminated under similar circumstances in other countries.

TABLE 11

Plants causing photosensitizations[a]

			(References)
Primary:			
Fagopyrum saggittatum	Buckwheat	Polygonaceae	*215*
Hypericum perforatum	St.-John's-wort, Klamath weed	Hypericaceae	*125*
Hepatogenic:			
Euphorbia maculata[b]	Milk purslane	Euphorbiaceae	*39*
Lantana sp.	Lantana	Verbenaceae	*177*
Nolina texana	Sacahuiste	Liliaceae	*144*
Tetradymia sp.	Horsebrush	Compositae	*10, 47, 76*
Tribulus terrestris	Puncture vine	Zygophyllaceae	*95*
Other:			
Agave lecheguilla	Lechuguilla	Amaryllidaceae	*142, 143*
Species of bluegreen algae[b, c]	Water bloom	(Cyanophyta)	*21*
Avena sativa[b]	Oats	Gramineae	*188*
Brassica napus[d, e]	Cultivated rape	Cruciferae	*39*
Species of fungi[c]	Molds	(Eumycophyta)	*85*
Medicago sativa[b]	Alfalfa	Leguminosae	*117*
Trifolium sp.	Clovers	Leguminosae	*27, 73*
Sorghum vulgare[b, c, f]	Sudan grass	Gramineae	*100*

[a] Various grasses suspected of causing photosensitizations in Texas *(197)*. A hepatogenic photosensitization from an unidentified plant has been reported from Vermont *(181)*. Frosted Bermuda grass also suspected in photosensitizations *(85)*. (See also Table 13.)

[b] See also Table 7.

[c] See also Table 13.

[d] See also Table 5.

[e] Also other Cruciferae *(39)*.

[f] See also Table 2.

TABLE 12

Plants which have caused severe mechanical injury

				(References)
Eremocarpus setegerus	Turkey mullein	Euphorbiaceae	Hairballs	*176*
Hordeum jubatum	Foxtail grass	Gramineae	Barbed awns	*78*
Indigofera hirsuta	Hairy indigo	Leguminosae	Superficial leg injury	*217*
Pisum sp.[a]	Pea straw hay	Leguminosae	Impaction	*111*
Rubus sp.	Blackberry vines	Rosaceae	Lodged in nasal passages	*167*
Setaria lutescens	Bristle grass	Gramineae	Mucous erosion from barbs	*6*

[a] See also Table 13.

Mechanical injury. A number of plants may inflict injury to animals in a purely mechanical manner. Usually obvious, they receive little attention; yet, particular plants under particular circumstances have caused economic loss sufficient to have been treated in the veterinary literature *(92)*.

Plants containing miscellaneous or unknown principles toxic to stock. The poisoning of human beings from milk of poisoned animals is practically nonexistent in this country, for the following reasons: The normal mechanism for the elimination of toxic principles from the animal body is by action of the liver or kidneys. Most toxic principles reach the milk in only minor amounts. Acutely poisoned animals usually either die or recover before many milking periods have elapsed. Milk flow may be reduced or completely stopped. Most dairy farmers throw out milk from animals exhibiting obvious symptoms of any kind. As a generalization, it may be pointed out that unless a toxic principle is concentrated in the milk, or unless the human species is much more susceptible to being poisoned by a given principle than is the lactating animal, the normal human diet (containing other foods) insures that the total intake of a poisonous principle will be below the toxic level. Finally, the present practice of pooling milk not only from many cows in a herd but from many herds in a relatively wide geographic area, insures that if a poisonous principle is present, it will be diluted far below the level of toxicity to the human being.

There are two kinds of poisonous principles that have caused poisoning in human beings in the past. In both situations, direct consumption of milk from a "family cow" was almost always involved. Low-grade poisonous principles with irritant action (such as volatile oils) may possibly cause the milk of animals ingesting them to become laxative or, further, to produce gastric distress in the human being. Cases are rare, minor, and difficult to diagnose. In the second instance, a unique poisonous principle contained, as far as is presently known, in only two members of the Compositae is involved. This is the higher alcohol, tremetol ($C_{16}H_{22}O_3$), which has been found in the geographically widely separated species *Aplopappus heterophyllus* and *Eupatorium rugosum (54)*.

Tremetol is unique in being concentrated in the milk of lactating animals, and since the symptoms in poisoned cattle are slow to develop and not particularly distinctive at first, highly toxic milk may be produced and ingested. It is common for symptoms to appear in a suckling calf before they do in the parent. Tremetol causes an upset in carbohydrate metabolism. In cattle, a prominent symptom is trembling following exercise and the disease is popularly termed "trembles." In human beings, poisoning from tremetol was common from Colonial times into the 19th Century and was known as "milk sickness." Symptoms included constipation, vomition, delirium, and often concluded in death. Unfortunately, for a long time the connection between these symptoms and the ingestion of milk was not known or only uncertainly suspected. Since a common treatment of the time for vomition was to give milk, its disastrous effects were multiplied. Entire villages were forced to move when their populations suffered severe mortality. The oft-reported case in which the death of Lincoln's mother (Nancy Hanks) was attributed to milk sickness may be mentioned again.

Moseley *(156)* has contributed a study of the history of milk sickness in this country and has examined the reasons for the tardiness in identifying the toxic principle and relating it to *Eupatorium rugosum.*

An extensive literature has developed in recent years concerning the toxicity of bracken fern (*Pteridium* sp.). This fern is common in many parts of the world and in wide areas of North America. It is responsible for stock loss of some magnitude. Aside from the economic interest thus engendered, bracken-fern poisoning has unique aspects that have intrigued various investigators. Briefly, the symptoms produced in single-stomached animals vary markedly from those in ruminant animals. In the former, the symptoms are those of B_1 avitaminosis. This condition has been demonstrated *(70)* to be brought about by the enzyme thiaminase present in bracken, which possibly effects cleavage of

TABLE 13 (Continued)

Plants containing miscellaneous or unknown principles toxic to stock[a]

			(References)
Acacia berlandieri	Guajillo	Leguminosae	*169*
Actinea sp.[b]	Pingue, bitterweed, rubberweed	Compositae	*16, 166*
Bluegreen algae[c, d]	Water blooms, pond scums	(Cyanophyta)	*56, 201*
Amsinkia intermedia[c]	Tarweed	Boraginaceae	*110, 150*
Aplopappus heterophyllus[e, i]	Rayless goldenrod	Compositae	*52*
Apocynum sp.	Dogbanes	Apocynaceae	*108*
Astragalus sp.[b]	Locoweeds	Leguminosae	*128, 137*
Astragalus decumbens[b]	Timber milk vetch	Leguminosae	*10*
Astragalus emoryanus[b]	Peavine	Leguminosae	*198*
Astragalus tetrapterus[b]	Poison vetch	Leguminosae	*102*
Baccharis ramulosa (= *B. pteronioides*)	Yerba-depasmo	Compositae	*127*
Baileya multiradiata	Baileya	Compositae	*139*
Centaurea solstitialis	Yellow star thistle	Compositae	*50*
Citrus sp.	Citrus	Rutaceae	*62*
Cynodon dactylon[g]	Bermuda grass	Gramineae	*4, 219*
Datisca glomerata	Durango root	Datiscaceae	*212*
Descurainia pinnata	Tansy mustard	Cruciferae	*95*
Drymaria sp.	Drymaria	Caryophyllaceae	*60, 140*
Equisetum sp.[h]	Horsetails	Equisetaceae	*89, 94, 173*
Eupatorium rugosum[i]	White snakeroot	Compositae	*156*
Eupatorium wrightii	Wright's eupatorium	Compositae	*96*
Flourensia cernua	Black brush, tarbush	Compositae	*147*
Gossypium sp.[j]	Cotton	Malvaceae	*184, 193, 194*
Gutierrezia microcephala[k]	Broomweed	Compositae	*61, 217*
Hordeum vulgare[c]	Barley	Gramineae	*43*
Karwinskia humboltiana	Coyotillo	Rhamnaceae	*135*
Lathyrus sp.[l]	Sweet peas	Leguminosae	*10*
Lippia ligustrina	White brush	Verbenaceae	*146*
Malus sylvestris	Apple	Rosaceae	*152*
Melia azedarach	Chinaberry	Meliaceae	*217*
Menziesia glabella	Rustyleaf	Ericaceae	*120*
Notholaena sinuata var. *cochisensis*	Jimmyfern	Polypodiaceae	*148*
Onoclea sensibilis	Sensitive fern	Polypodiaceae	*214*
Ornithogalum umbellatum	Star of Bethlehem	Liliaceae	*172*
Oxytenia acerosa	Oxytenia	Compositae	*206*
Oxytropus sp.[f]	Locoweeds	Leguminosae	*10*
Persea americana	Avocado	Lauraceae	*103*
Phoradendron sp.	Mistletoe	Loranthaceae	*224*
Phyllanthus abnormis	Spurge	Euphorbiaceae	*149*

TABLE 13 (Concluded)

Pisum arvense[m]	Austrian pea forage	Leguminosae	*175*
Pisum sativum[m]	Canning pea vines	Leguminosae	*222*
Psilostrophe sp.	Paperflower	Compositae	*102, 141*
Pteridium sp.[b]	Bracken fern	Polypodiaceae	*70*
Quercus sp.	Scrub oaks	Fagaceae	*132*
Rudbeckia lacineata	Goldenglow	Compositae	*190*
Salvia lanceolata	Annual sage	Labiatae	*10*
Sartwellia flaveriae	Sartwellia	Compositae	*145*
Sium suave	Water parsnip	Umbelliferae	*229*
Solidago spectabilis	Western goldenrod	Compositae	*74*
Stipa vaseyi	Sleepygrass	Gramineae	*124*
Triticum sp.[c]	Wheat pasture	Gramineae	*171, 187*
Vicia villosa	Hairy vetch seed	Leguminosae	*45*
Xanthium sp.[n]	Cockleburr	Compositae	*113*
Zea mays[c]	Corn	Gramineae	*31, 164, 189*
Zephyranthes atamasco	Atamasco lily	Amaryllidaceae	*33*

[a] Several suspected of toxicity as the result of mold or other fungus activity. (See also Table 11.)
[b] Also called *Hymenoxys.*
[c] See also Table 7.
[d] See also Table 11.
[e] Also called *Haplopappus.* (See also Table 8.)
[f] Certain species of *Astragalus* are toxic by reason of their selenium *(q.v.)* content. Others are locoweeds. A third group of species is not known to be toxic. At least three species are toxic, containing toxic principles that are neither selenium nor those producing symptoms typical of loco *(102)*. Two of these species exhibit a correlation between toxicity and geological formation, yet neither contains selenium nor produces symptoms characteristic of selenium poisoning *(11, 198)*. (See also "alkaloids".)
[g] Frosted Bermuda grass also suspected in photosensitization.
[h] Contains an enzymic antithiamine principle toxic to nonruminants.
[i] Contains tremetol, a higher alcohol, as the toxic principle.
[j] Contains gossypol in press cake as the toxic principle.
[k] See also Table 8.
[l] *Lathyrus* has been intensively investigated as the source of a physiologically active factor *(151)*. It has caused European, and been suspected of domestic, stock loss.
[m] See also Table 12.
[n] Contains hydroquinone as the toxic principle.

the thiamine molecule at the methylene bridge between the pyrimidine and thiazole parts. Administration of thiamine brings about recovery in cases of bracken poisoning in horses and its administration together with a bracken diet prevents symptoms from appearing in rats.

The situation in cattle is more complex. It has been reviewed by Evans *et al. (69)*. The primary lesion is a degenerative change in the blood manufacturing bone marrow, requiring a number of weeks to develop and resulting in a thrombocytopenia and leucopenia *(160, 162)*. Injection of thiamine has little or no beneficial effect, and no shortage of thiamine can be demonstrated in the animal. Experimental evidence *(161, 202)* suggests that a specific poison is responsible for the deleterious reaction in the marrow. Recently, suggested by its efficacy in treating human patients suffering from irradiation leucopenia, DL-batyl alcohol was used and proved effective as a treatment in experimentally induced cases of bracken poisoning in cattle *(203)*. A simple blood test for bracken poisoning is described by Burnside *(30)*, from work of Naftalin and Cushnie.

Various species of Horsetail *(Equisetum)* have been known to be toxic to stock in this country for a long time *(89, 173)*. A similarity in symptoms between poisonings by *Equisetum* and *Pteridium* in horses prompted an investiga-

tion of the former for an antithiamine factor. Its presence has been demonstrated and its enzymatic nature established *(94)*.

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DIFFERENCES IN THE ABILITY OF LACTIC STREPTOCOCCI TO FORM ALDEHYDES FROM CERTAIN AMINO ACIDS

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SUMMARY

Resting cells of five cultures of lactic streptococci were examined as to their ability to form flavorful aldehydes from four amino acids. Two strains of *Streptococcus cremoris*, two strains of *Streptococcus lactis*, and one strain of *Streptococcus lactis* var. *maltigenes* were incubated with isoleucine, valine, methionine, and phenylalanine, and the resulting aldehydes were isolated and identified as their 2,4–dinitrophenylhydrazones. The incubated mixtures of the amino acids and *S. lactis* var. *maltigenes* cells were the only ones in which 2-methylbutanal, 2-methylpropanal, 3-methylthiopropanal, and phenylacetaldehyde accumulated in sufficient quantities to allow isolation and identification of their derivatives. One of the strains of *S. lactis* formed trace quantities of these aldehydes; whereas, neither strain of *S. cremoris*, nor the freshly isolated strain of *S. lactis*, appeared to degrade the amino acids to aldehydes. The activity of the alpha-keto acid decarboxylase system present in some lactic streptococci and its possible role in the development of Cheddar cheese flavor are discussed.

Streptococcus lactis and *Streptococcus lactis* var. *maltigenes* are known to possess a transaminase system which effects the transfer of the amino group of leucine to alpha-ketoglutaric acid, resulting in the formation of glutamic acid and alpha-ketoisocaproic acid *(13)*. *S. lactis* var. *maltigenes* possesses, in addition, an enzyme which decarboxylates this keto acid to 3-methylbutanal, the compound responsible for the typical aroma and flavor of milk cultures of this variant *(14)*. *S. lactis* cells, on the other hand, produce little or no 3-methylbutanal from the keto acid *(13)*.

Keeney and Day *(11)* suggest that the Strecker degradation of amino acids yielding various aldehydes may play a prominent role in Cheddar cheese flavor development. Bassett and Harper *(2)* reported the presence of pyruvic and alpha-ketoglutaric acids and trace quantities of oxalacetic and alpha-ketoisocaproic acids in Cheddar cheese. They also found small quantities of acetone, acetaldehyde, and a five-carbon carbonyl compound.

The foregoing reports have prompted an investigation of the abilities of various organisms associated with the ripening of Cheddar cheese to convert certain amino acids to flavorful aldehydes. The present report contributes further information on aldehyde formation from isoleucine, valine, methionine, and phenylalanine by cells of *S. lactis* var. *maltigenes*, as well as by two non-malty strains of *S. lactis* and two strains of *S. cremoris*.

EXPERIMENTAL PROCEDURE

Cultures. Two strains of *S. lactis*, designated as L_2 and L_r, two strains of *S. cremoris*, C_{14} and C_8, and one strain of *S. lactis* var. *maltigenes*, designated as M_1, were employed as the test cultures throughout this experiment. These

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cultures were transferred monthly in sterile homogenized milk and held at 4° C. after incubation. L_r and C_8 were recent isolates from raw milk and buttermilk starter, respectively; whereas, L_2, C_{14}, and M_1 were strains from the laboratory stock culture collection.

Preparation of cell-substrate suspensions. Two-liter lots of trypticase broth (BBL) supplemented with 20 mg. of pyridoxine · HCl were inoculated with 50 ml. of 18-hr. broth cultures of each of the test organisms and incubated at 32° C. for from 24 to 48 hr. The test cultures of *S. cremoris* required 48 hr. of incubation to produce cell yields comparable to the 24-hr. cultures of *S. lactis* and *S. lactis* var. *maltigenes*. At the end of the incubation period, the cells were collected by centrifugation for 10 min. in a high-speed angle centrifuge at 12,800 × G. The cells were washed twice with distilled water and suspended in 50 ml. of 0.33 *M* phosphate buffer at pH 7.0 *(19)*. The buffered suspensions were added to 100-ml. quantities of buffer to which had been added 0.2 mg. of DL-amino acids under examination. Comparable buffered cell suspensions containing no added amino acid were prepared as controls.

Detection, isolation, and identification of aldehyde derivatives. The cell–amino acid mixtures and the controls contained in tightly stoppered glass bottles were incubated at 32° C. for 3 hr., then they were opened and the presence of characteristic odors recorded. The cells were removed by centrifugation, 20 ml. of 0.2% DNP[1] hydrazine in 2*N* HCl added to each supernatant, and the mixtures allowed to stand at room temperature for 2 hr. When isoleucine or valine was the test substrate, the DNP hydrazones were extracted exhaustively with carbonyl-free petroleum ether and in all other cases the extracting solvent was benzene-heptane (1 : 1 by volume). The extracts were evaporated to dryness on a rotating flash evaporator operated at a temperature not exceeding 40° C. The derivative residues were taken up in a minimum of benzene-heptane solvent and those which had been extracted initially with benzene-heptane were freed from excess DNP hydrazine by passage through a column of anhydrous magnesium sulfate, as described by Jackson and Morgan *(9)*. The derivative solvents were adjusted to 1 to 2 ml. and spotted in amounts ranging from 0.01 to 0.08 ml. on Whatman No. 1 paper, along with known DNP hydrazones of acetaldehyde, acetone, and aldehydes corresponding to the test amino acids. Papers prepared in this manner were developed by the method of Heulin *(7)*.

When the chromatographic analyses indicated the presence of sufficient quantities of the derivatives of the amino acid–derived aldehydes, the extracts were evaporated to dryness and fractionated by column chromatography, using either cellulose or celite–silicic acid as the adsorbents *(15, 18)*. The derivative residues resulting from the degradation of isoleucine and valine were taken up in a minimum of methanol-saturated heptane and run into 28- by 300-mm. cellulose columns, previously prepared by the method described by Morgan *et al. (15)*. Elution was continued with methanol-saturated heptane until the fastest moving band in each column had been collected. If the bands appeared to be pure when chromatographed on paper, and if they ran parallel with known derivatives of

[1] DNP is used throughout this paper as an abbreviation for 2,4–dinitrophenyl.

2-methylbutanal or 2-methylpropanal, the solvent was evaporated from the fractions, the derivatives recrystallized three times from 80% ethanol, and the melting points determined.

The derivative residues obtained from the cell suspensions containing methionine were taken up in 10 ml. of chloroform and added to a dry 28- by 150-mm. celite–silicic acid column, prepared by the method of Gordon *et al. (6)*. After the chloroform extract had been adsorbed, 50 ml. of carbonyl-free petroleum ether was allowed to percolate into the column. When the petroleum ether had been adsorbed, 250 ml. of the developing solvent (6% ethyl ether in petroleum ether) was added for the separation of the derivatives of acetaldehyde and 3-methylthiopropanal *(18)*. The 3-methylthiopropanal separated as a compact band running behind that of acetaldehyde. This band was eluted and evaporated to dryness at room temperature. Melting point determinations were made on the resulting crystalline derivative.

By passage of the derivative mixture from the cell suspensions containing phenylalanine through a celite–silicic acid column with a developing solvent of 2% ethyl ether in petroleum ether, it was possible to separate the phenylacetaldehyde derivative from the small amounts of acetaldehyde and acetone derivative present. Melting-point determinations on the isolated derivative were made after recrystallization from ethanol.

If only trace amounts of the specific amino acid–derived aldehyde derivatives were present in extracts from cell suspensions as indicated by the size and intensity of the spots on the chromatograms, as well as the amount of the derivative extract necessary to produce such spots, the extracts were evaporated to dryness and the aldehydes present regenerated by the addition of levulinic acid and the application of heat *(10)*. The detection of characteristic aromas from this test was considered confirmatory evidence for the presence of the various aldehydes.

All melting-point determinations were made on a Koffler micro-melting-point apparatus. The micro-sublimations were made on the same instrument, by the procedure described by Morgan *et al. (15)*.

RESULTS

Paper chromatograms of the DNP hydrazones of the neutral carbonyl compounds indicated that there were traces of acetaldehyde and/or acetone in all the incubated buffered cell preparations. Such chromatograms also indicated that cells of neither of the *S. cremoris* cultures, C_{14} and C_8, or the recently isolated *S. lactis* culture, L_r, could effect degradation of isoleucine to 2-methylbutanal, valine to 2-methylpropanal, methionine to 3-methylthiopropanal, or phenylalanine to phenylacetaldehyde. None of the cell suspensions of C_{14}, C_8, and L_r, incubated with the various test amino acids, possessed aromas typical of the several aldehydes.

The cells of *S. lactis* L_2 possessed a limited ability to degrade the amino acids to aldehydes yielding only trace amounts of 2-methylbutanal, 2-methylpropanal, 3-methylthiopropanal, and phenylacetaldehyde from the related amino acids.

Traces of the derivatives of the first two aldehydes were detectable on paper chromatograms, but practically the entire derivative extract from the suspensions of L_2 cells incubated with isoleucine and valine had to be spotted on the paper to obtain definitive spots of 2-methylbutanal and 2-methylpropanal DNP hydrazones.

The typical cooked cabbage–like odor of 3-methylthiopropanal and the flower-like aroma of phenylacetaldehyde were detectable over the cell suspensions of L_2 incubated with methionine and phenylalanine, respectively. Positive detection of the trace quantities of the derivatives of these aldehydes was not possible by paper chromatography because the derivatives of acetaldehyde and 3-methylthiopropanal are inseparable, as is the case with those of phenylacetaldehyde and acetone. However, when the respective derivative extracts were heated with levulinic acid, the typical odors of 3-methylthiopropanal and phenylacetaldehyde were taken as an indication of the presence of the regenerated aldehydes.

S. lactis var. *maltigenes* was the only strain of the five cultures of lactic streptococci that possessed the ability to effect sufficient degradation of the four test amino acids to allow isolation and crystallization of the DNP hydrazones of the respective aldehydes. The incubated mixture of isoleucine and M_1 cells had a pronounced odor resembling that of 2-methylbutanal. The DNP hydrazone isolated from this mixture crystallized in the form of orange-yellow plaques which melted from 129 to 131° C. after the third recrystallization from ethanol. These plaques sublimed at 130° C. and the sublimate melted from 130 to 131.5° C. When sublimed with a known sample of 2-methylbutanal derivative, the mixed sublimate melted from 129 to 131° C. These melting points are in accord with those reported in the literature *(1, 9)*.

The mixture of M_1 cells and valine developed a strong odor resembling that of 2-methylpropanal during incubation. The derivative isolated from this mixture crystallized from ethanol in the form of yellow needles, which melted from 184–185.5° C. These crystals sublimed from 180 to 181.5° C. in the form of hexagonal plates. A sublimate from a mixture of the isolated derivative and an equal amount of a known derivative of 2-methylpropanal, with a melting range of 185-186° C., melted from 185 to 187° C. These melting points are in agreement with those reported by Bryant *(4)*.

A strong odor resembling that of 3-methylthiopropanal emanated from the mixture of M_1 cells and methionine. The major fraction isolated from the derivative extract from this mixture yielded crystals which melted from 124 to 125.5° C. This melting range was higher than that reported in the literature *(8, 16, 17)* but agreed closely with that of a known derivative of 3-methylthiopropanal (123–124° C.) prepared from methionine by oxidation with ninhydrin. A mixture of these derivatives melted from 122 to 125° C.

The incubated mixture containing phenylalanine and *S. lactis* var. *maltigenes* cells possessed a pleasant, flower-like odor which was indistinguishable from that of a sample of phenylacetaldehyde prepared in the laboratory, by treatment of phenylalanine with isatin. The derivative isolated from this mixture recrystallized from ethanol as golden leaflets and melted from 121 to 122° C., with no

apparent sublimation. The derivative of authentic phenylacetaldehyde prepared in the laboratory melted over the range of 115–120° C. Two different melting points for the DNP hydrazone of phenylacetaldehyde are given in the literature as 110° C. *(3)* and 121° C. *(5)*. This may be indicative of the existence of two forms of the derivative and may account for the melting behavior of the authentic hydrazone.

DISCUSSION

The present investigation has demonstrated that resting cells of *S. lactis* var. *maltigenes* are able to convert the respective amino acids to 2-methylbutanal, 2-methylpropanal, phenylacetaldehyde, and 3-methylthiopropanal. Accumulation of the latter three aldehydes in milk cultures of this organism was not demonstrated by Jackson and Morgan *(9)*. Had 2-methylpropanal been present in the distillates from their milk cultures, it would have been detected readily on the paper chromatograms of the DNP hydrazone extracts. The presence of phenylacetaldehyde and 3-methylthiopropanal in such distillates could have been overlooked because of the inability of this chromatographic technique to separate the derivative of the former from that of acetone and the latter from that of acetaldehyde. However, if these aldehydes were involved extensively in the typical malty aroma of *S. lactis* var. *maltigenes* milk cultures, it is doubtful that this aroma could be simulated by the simple addition of 3-methylbutanal to milk *(9)*.

The ability of the cells of the *S. lactis* culture, L_2, to produce traces of aldehydes from amino acids and the apparent inability of the cells of the L_r culture to do so, suggests that among nonmalty strains of *S. lactis* there exists a variation in the degradative ability responsible for the conversion of amino acids to aldehydes. In the case of definitely malty strains of *S. lactis* (var. *maltigenes*), this ability has apparently developed to a high degree.

Since alpha-ketoglutaric acid and several free amino acids have been demonstrated in Cheddar cheese rather early in the ripening period *(2, 12)*, transaminases present in starter-culture cells and/or cells of *S. lactis* present in raw milk may convert some of these free amino acids to alpha-keto acids. The keto acids thus formed may or may not be converted to the corresponding aldehydes, depending upon the presence of active decarboxylases in cells of *S. lactis* and/or other organisms in the cheese.

If the aldehydes derived from certain amino acids are involved in typical Cheddar cheese flavor, as suggested by Keeney and Day *(11)*, then an investigation of the use of *S. lactis* strains possessing keto acid decarboxylase as cheese starters will be warranted.

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EFFECT OF PASTEURIZATION AND COOL-AGING ON THE SALT BALANCE IN MILK

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SUMMARY

The effects of pasteurization and cool-aging on the distribution of calcium, magnesium, phosphorus, and citric acid in milk into curd and whey were studied by analyzing these constituents in rennet whey from milk after the appropriate treatments. Statistical analysis of the data from ten bulk-milk samples revealed that the differences were not significant, except with respect to magnesium in curd from cool-aged milk. These variations, however, did not reflect any significant change in the Ca:P ratios in the curd. The results were not in harmony with the observations on pure salt solution under similar conditions, and the variations in the behavior of milk are considered partly due to the colloidal systems present in milk. The importance of considering magnesium and citric acid simultaneously is pointed out in any attempt to explain the ionic equilibria regarding calcium phosphate in milk.

The ionic equilibria in milk are influenced by heat treatments, as exhibited by the changes in pH on pasteurization and heating *(1, 9)*, the rate of exchange of anions and cations to ion exchange resins *(2)*, the rennet coagulation of milk *(13)*, the alcohol test *(19)*, the heat coagulation of milk *(16, 18)* and, possibly, other phenomena. The criteria for the evaluation of the influence of heat in the various phenomena are not clear-cut, and the problem is rendered more difficult by several overlapping effects, as well as by the same effect being observed under several varied conditions. The direct measurement of the changes in a heterogeneous system like milk is difficult, but several methods have been used whereby valuable information has been obtained regarding the influence of heat on milk. Thus, Mattick and Hallet *(13)* observed that rennet coagulation of milk heated to temperatures ranging from 40 to 100° C. became progressively slower on cool-aging. They followed the effect of cool-aging on rennet hysteresis of milk for only 5 hr. after heating and the inference has been quoted that equilibrium tends to be approached in 5 hr. Albrecht *(1)* extended these studies over a period of 24 hr. and found that significant changes occurred beyond 5 hr., and that about 24 hr. of cool-aging were required to approach equilibrium.

The influence of heat on ionic equilibria in milk has been studied by using the resin exchange technique of Baker and Gehrke *(2)*, who did not observe any effect of heating for 30 min. at temperatures ranging from 40 to 80° C. However, the exchangeable calcium was significantly decreased at temperatures from 100 to 120° C. for a holding time of 30 min. Further decreases were observed with longer holding periods. The alteration in the exchangeability of calcium was found to be permanent and did not revert on cool-aging at 5° C. for 48 hr. It appears that this method is not sensitive to changes occurring in the mineral

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balance due to heat treatments during normal pasteurization of milk. Hilgeman and Jenness *(10)* used collodion membranes in their ultrafiltration studies and reported a decrease in the dissolved calcium and phosphorus on heating milk at 80° C. for 30 min. They also found that there was a reversal of this effect on cool-aging for 48 hr. at 5° C. Daum *(4)* heated milk from 106 to 110° for 6 min. and also observed that the calcium decreased in the ultrafiltrate upon standing after heating.

The similarities of heat coagulation of milk and rennet coagulation have been indicated by Pyne and McHenry *(16)*, in that both involve alterations which render the casein more sensitive to precipitating action of calcium ions. It may further be noted that the presence of calcium is essential for clot formation by rennet and also accelerates clotting by heat. The importance of heat treatment on the ionic equilibria in milk and its interrelations with other constituents thus are amply illustrated. It was planned to study the effect of pasteurization and cool-aging on the minerals in whey to gain further insight into possible effects and mechanisms involved in the rennin coagulation process and in the alteration of the ionic equilibria in milk.

EXPERIMENTAL PROCEDURES AND METHODS

The study of the effects of heat was confined to pasteurization temperatures (143° C. for a holding period of 30 min.) and the holding process was used because the conditions for commercial pasteurization by the high-temperature-short-time process are not easily reproduced in the laboratory. Pasteurization was accomplished by using the thermostatically controlled laboratory pasteurizer described by Albrecht *(1)*. This provided for four water baths, each equipped with an agitator to circulate the heating water, a steam inlet for rapid heating, and an electric heating element with Fenwal thermoswitch for accurate control of temperature during holding process. The aluminum vessels to hold the samples were equipped with paddle agitators. For cool-aging, the samples were stored in a refrigerator at 45° F. after cooling them rapidly in an ice bath. The raw milk samples, the same after pasteurization and the same after the pasteurization and cool-aging for 24 hr., were analyzed simultaneously for total and whey-soluble calcium, magnesium, phosphorus, and citric acid. Raw bulk samples of milk were obtained from the commercial plant of the Department of Dairy and Food Industries. The procedure for preparation of whey and the methods of analyses for calcium, magnesium, phosphorus, and citric acid, were the same as described earlier *(21)*.

RESULTS AND DISCUSSION

The analyses for total and whey-soluble calcium, magnesium, phosphorus, and citric acid were carried out on ten samples and the results are presented (Table 1), giving the distribution of these constituents under the different treatments. From the data (Table 1), the amounts of calcium, magnesium, phosphorus, and citric acid in the corresponding curd were computed according to a procedure described elsewhere *(21)*, to get an estimate of these constituents in the colloidal phase. Further, the ratios of Ca : P in the colloidal part of the

TABLE 1

Influence of pasteurization and cool-aging on the distribution of calcium, magnesium, phosphorus, and citric acid in milk

Sample	Calcium				Magnesium				Phosphorus				Citric Acid			
		Soluble in				Soluble in				Soluble in				Soluble in		
	Total	Raw	Past.	Aged	Total	Raw	Past.	Aged	Total	Raw	Past.	Aged	Total	Raw	Past.	Aged
	(mg/100 ml. milk)															
1	174.8	76.2	73.9	.....	14.1	9.9	10.6	7.9	98.5	36.5	35.8	33.6	134.9	116.8	125.1	125.4
2	133.5	50.7	51.9	61.3	10.4	7.6	8.1	7.8	100.0	25.5	35.0	32.7	157.1	135.9	146.2	120.9
3	129.0	53.3	55.6	55.6	10.2	8.4	7.3	7.3	122.0	43.2	39.7	30.5	155.9	142.8	150.9	154.9
4	116.4	41.3	41.9	42.5	10.8	6.0	7.3	7.8	70.0	30.4	33.7	31.4	153.4	150.8	143.2	143.5
5	120.8	41.6	41.9	40.1	10.2	6.5	6.0	7.9	75.0	28.0	35.3	37.0	153.5	149.1	142.4	151.5
6	126.1	30.8	41.4	42.2	9.6	8.0	6.8	4.1	91.5	40.2	40.0	39.8	152.7	131.5	142.6	141.2
7	124.7	45.6	45.9	42.2	9.9	8.7	7.6	4.4	90.6	41.7	41.3	40.7	169.6	153.8	153.1	158.0
8	132.4	59.8	51.4	45.9	10.3	7.3	6.6	5.1	93.6	42.6	41.6	41.3	171.2	157.8	154.3	137.4
9	116.4	44.6	39.3	50.8	10.4	8.4	6.2	5.4	80.8	38.7	37.4	39.1	160.0	154.5	143.2	144.0
10	123.8	50.0	40.6	47.7	11.3	7.3	8.5	6.6	84.8	41.1	38.8	53.1	171.2	163.4	157.4	151.5
Av.	124.8[a]	46.4[a]	45.5[a]	47.6[a]	10.7	7.8	7.5	6.4	90.7	36.8	37.9	36.9	158.0	145.6	145.8	142.8
Per cent of total	.....	37.2	36.5	38.1	.....	72.9	70.0	59.8	.....	40.6	41.8	40.7	.....	92.2	92.3	90.4

[a] Sample No. 1 not included.

TABLE 2

Distribution of colloidal calcium, magnesium, citric acid, and phosphorus; and Ca:P ratios in the curd as influenced by pasteurization and cool-aging of milk

Sample	Calcium			Magnesium			Citric Acid			Phosphorus			Ca:P ratios		
	Raw	Past.	Aged	Raw	Past.	Aged	Raw	Past.	Aged	Raw	Past.	Aged	Raw	Past.	Aged
	(mM/100 ml. milk)														
1	1.84	1.89	.....	0.17	0.14	0.26	0.14	0.08	0.07	1.42	1.44	1.51	1.32	1.35	.....
2	1.44	1.41	1.18	0.12	0.09	0.11	0.17	0.09	0.28	1.82	1.52	1.59	0.76	0.93	0.64
3	1.26	1.21	1.21	0.07	0.12	0.12	0.10	0.04	0.01	1.96	2.07	2.37	0.63	0.62	0.56
4	1.25	1.23	1.22	0.20	0.14	0.12	0.02	0.08	0.08	0.70	0.60	0.67	2.04	2.15	1.88
5	1.35	1.34	1.39	0.15	0.17	0.09	0.03	0.09	0.02	0.94	0.70	0.65	1.56	2.03	2.25
6	1.75	1.49	1.47	0.07	0.12	0.23	0.17	0.08	0.09	1.07	1.08	1.09	1.54	1.42	1.48
7	1.35	1.34	1.43	0.05	0.09	0.23	0.12	0.13	0.09	1.00	1.01	1.03	1.28	1.29	1.52
8	1.19	1.40	1.53	0.12	0.15	0.21	0.11	0.13	0.26	1.07	1.10	1.11	1.12	1.29	1.33
9	1.17	1.30	1.01	0.08	0.17	0.21	0.04	0.13	0.13	0.78	0.82	0.77	1.55	1.63	1.42
10	1.22	1.45	1.27	0.17	0.12	0.19	0.06	0.11	0.15	0.83	0.90	0.44	1.60	1.62	2.98[a]
Av.	1.38	1.41	1.30	0.12	0.13	0.18	0.096	0.096	0.118	1.159	1.124	1.123	1.340	1.433	1.385

[a] Sample 10 not included in the average for the Ca:P ratio of the cool-aged milk.

rennet curd were also computed according to Verma and Sommer *(21)* and are shown (Table 2).

The ten samples studied here showed wide variations in respect to the constituents analyzed, and the averages of the values do not reflect any statistical differences under the various treatments, except in the case of magnesium, where significant differences were observed. There was a significant increase in the curd-magnesium in the cool-aged lot as compared to raw and pasteurized ones. However, the magnitude of these variations was such that it did not reflect in any alterations in the Ca : P ratios in the curd. Individual samples do show differences in the Ca : P ratios in the curd as well as in the whey-soluble constituents studied here. This caution should be kept in mind, along with the fact that the statistical insignificance of these variations may not be of much help in any efforts to evaluate and predict the behavior of any particular milk under other processing conditions. The variations in pH of milk are illustrative of this in the sense that alterations in pH, too small to be statistically significant, may cause rather profound changes in the characteristics of milk.

The Ca : P ratios in the colloidal phase of milk are often worked out without accounting for the presence of magnesium and citric acid. When the ratios of magnesium/citric acid were worked out for the curd (Table 2), they were found to be 1.25 for raw milk, 1.35 for pasteurized milk, and 1.53 for the pasteurized and cool-aged milk. It is not to be suggested in these ratios that magnesium citrate is formed in the curd with the exclusion of other possibilities, but it clearly indicates that there are alterations in the equilibria involving these constituents and they can not be ignored completely in any careful consideration of the inorganic equilibria, and comparisons arrived at by considering merely the calcium and phosphorus will be erroneous. Thus, in an empirical manner we found that the Ca : P ratios for inorganic phase of curd should be corrected by using a factor of 1.125 for the ratios in raw milk, 1.143 for pasteurized milk, and 1.196 for cool-aged milk, if they have been computed without taking magnesium and citric acid into account. These correction factors may not be applicable to all samples of milk and are included merely to demonstrate the usual discrepancy when magnesium and citric acid are ignored in the calculations of the Ca : P ratios in milk. Due to the many assumptions that enter into these calculations, and because of the wide variations observed from sample to sample, there is agreement with the view of Ter Horst *(20)* that the average Ca : P ratios have no special significance, inasmuch as they may not be used as absolute measures of the state of calcium phosphate in milk.

The presence of tricalcium phosphate in the native raw milk has been demonstrated by Pyne *(14)*, Ling *(11)*, van der Burg *(3)*, Pyne and Ryan *(17)*, and Evenhuis *(7)*. It has been further supported by the work of Ford, Ramsdell, and Landsman *(8)* and of Edmondson and Tarassuk *(6)*. An important role is played by this constituent in milk, and the study of a pure salt solution prepared in accordance with the composition of salts in milk serum demonstrated the role of the di- and tribasic citrates and/or phosphates of calcium and/or magnesium in alterations in pH and Ca :P ratios on heating and cool-aging

Sample 10 not included in the average for the Ca:P ratio of the cool-aged milk.

(22). The results of the investigation on the whey-soluble constituents in this study under pasteurization and cool-aging are not in harmony with the results on pure salt solution and do not lend a clear-cut support to the observations of Magee and Harvey *(12)*, Mattick and Hallet *(13)*, and others *(10)* on the condition of diffusible calcium and phosphorus studied by using collodion membranes. However, the results support the ion exchange studies of Baker and Gehrke *(2)* who did not observe any variations in exchangeable calcium on heating milk for 30 min. at temperatures of 40–80° C.

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(Statistical Appendix)

TABLE 1

Variance analysis[a] *of calcium, magnesium, citric acid, phosphorus, and Ca:P ratios in rennet curd with test of significant differences*[a] *between raw, pasteurized, and cool-aged milks*

Components	Sources of variance	Degrees of freedom	Mean squares	
			Between	Within
Calcium	Raw pasteurized, and aged	2	0.0280	0.0403
	Raw *vs.* pasteurized and aged	1	0.0040	0.0403
	Pasteurized *vs.* aged	1	0.0520	0.0403
Magnesium	Raw, pasteurized, and aged	2	0.0091[b]	0.0024
	Raw and pasteurized *vs.* aged	1	0.0177[c]	0.0024
	Pasteurized *vs.* raw	1	0.0006	0.0024
Citric Acid	Raw, pasteurized, and aged	2	0.0016	0.0041
	Raw and pasteurized *vs.* aged	1	0.0032	0.0041
	Pasteurized *vs.* raw	1	0.0001	0.0041
Phosphorus	Raw, pasteurized, and aged	2	0.0042	0.2376
	Raw *vs.* pasteurized and aged	1	0.0084	0.2376
	Pasteurized *vs.* aged	1	0.0001	0.0001
Ca:P ratio	Raw, pasteurized, and aged	2	0.0216	0.2299

[a] According to Snedecor, G. W. *Statistical Methods.* 4th ed. Iowa State College Press, Ames. 1946.

[b] Significant with p less than 0.05.

[c] Highly significant with p less than 0.01 and more than 0.025.

SECONDARY ALCOHOLS IN BLUE CHEESE AND THEIR RELATION TO METHYL KETONES

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SUMMARY

Neutral noncarbonyl volatiles isolated from Blue cheese were characterized by gas and paper chromatography as being pentanol-2, heptanol-2, and nonanol-2. Results from metabolic studies with *Penicillium roqueforti*, and from the examination of volatiles produced at different stages in the ripening of Blue cheese, suggest that the secondary alcohols are formed by a reduction of the ketones pentanone-2, heptanone-2, and nonanone-2. The qualitative value of plotting retention times, obtained on two different stationary phases, was extended by a technique which may enable the estimation of boiling points of unknowns within a given class.

Previous investigators have been concerned with neutral and acidic carbonyl compounds in Blue cheese *(1, 5, 6)*. The use of gas chromatography has indicated that additional neutral components are present in this mold-ripened cheese. This investigation was done to characterize some of the components and to determine their relation to the ripening process in Blue cheese.

EXPERIMENTAL PROCEDURE

Isolation and concentration of neutral Blue cheese volatiles. During this study, approximately 36 lb. of typical Danish Blue cheese, representing three different lots of cheese, was used in isolating the volatiles of interest.

Successive 2-lb. samples of cheese were mixed with distilled water in a Waring blendor, then subjected to steam distillation. One liter of distillate was collected from each distillation. The aqueous distillate was adjusted to the phenolphthalein end-point with sodium carbonate and saturated with sodium chloride before being extracted twice with 150-ml. aliquots of peroxide-free di-ethyl ether.

The volatiles were concentrated by cautious evaporation of the ether extract in the presence of anhydrous calcium sulfate. The resulting concentrate possessed the characteristic aroma of Blue cheese.

A vacuum distillation was carried out on an aqueous slurry of Blue cheese, to insure that the components found in the atmospheric steam distillate were not formed as a result of heating. The temperature during the distillation did not exceed 35° C. Dry ice acetone traps were used to collect the volatiles.

Chromatographic separation of components. The neutral volatiles from Blue cheese that had been concentrated in ether were separated by gas chromatography, using a modified Perkin-Elmer 154 Vapor Fractometer. A typical gas chromatogram is shown (Figure 1). The elution of Peaks 1, 4, and 6 into a 2,4-dinitrophenylhydrazine solution (0.2% in 2 *N* HCl) indicated that they con-

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tained a carbonyl group. Retention times of these components on separate columns, having Carbowax 1500 and di-*N*-decyl phthalate as stationary phases, indicated that Peaks 1, 4, and 6 represented pentanone-2, heptanone-2, and nonanone-2, respectively. These ketones were isolated and identified previously from Blue cheese by Patton *(6)* and Morgan *(5)*. Peaks 3, 5, and 8 gave a negative test with 2,4-dinitrophenylhydrazine. The vacuum distillate contained all the components shown to be present in the atmospheric distillates.

Further characterization of Peaks 3, 5, and 8 was accomplished by utilizing the differential retention times of known classes of compounds on two different stationary phases. Peaks 3, 5, and 8 were collected after separation on the carbowax column, by employing an ether trap cooled by a dry ice acetone bath. Their retention times along with known compounds are shown (Figure 2). Logarithms of the retention times were plotted so as to obtain parallel lines for a series of homologous compounds. The odor and retention times of the unknown components 3, 5, and 8 suggested that they were alcohols.

By extending lines perpendicular to the ordinate, from the retention times of the iso-alcohols—2-methyl-propanol and 3-methyl-butanol—to the intercepts of the slope of the normal alcohols, a relationship between boiling points and retention times was evident.

Referring to Figure 2, this technique was applied to the unknown components 3 and 5 by taking the ratio of the distances B/A times the difference in boiling points of the nearest known alcohols, and adding this to the alcohol having the lower boiling point. The boiling point of Component 5 was calculated as 161° C. and of Component 3 as 121° C.

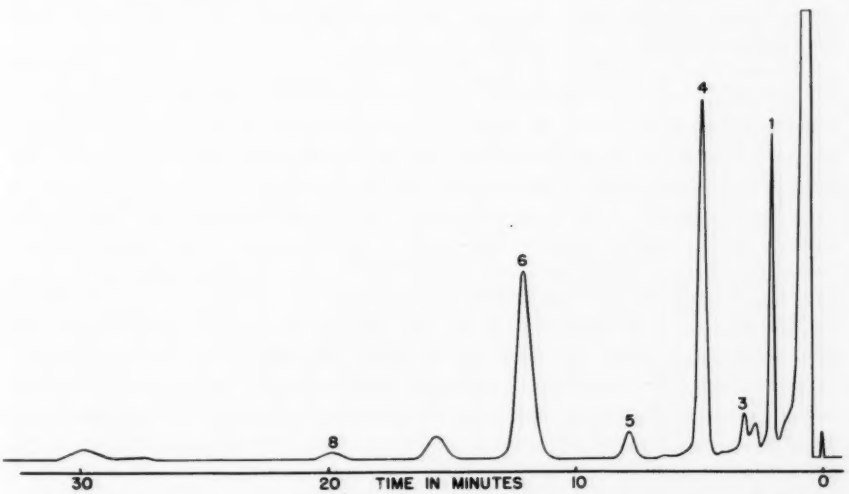

FIG. 1. Gas chromatogram of the ether extract of neutral volatiles from Danish Blue cheese. Temperature 125° C., 5 m.v. span, flow rate 160 cc. per min., column Carbowax 1500—2-meter length.

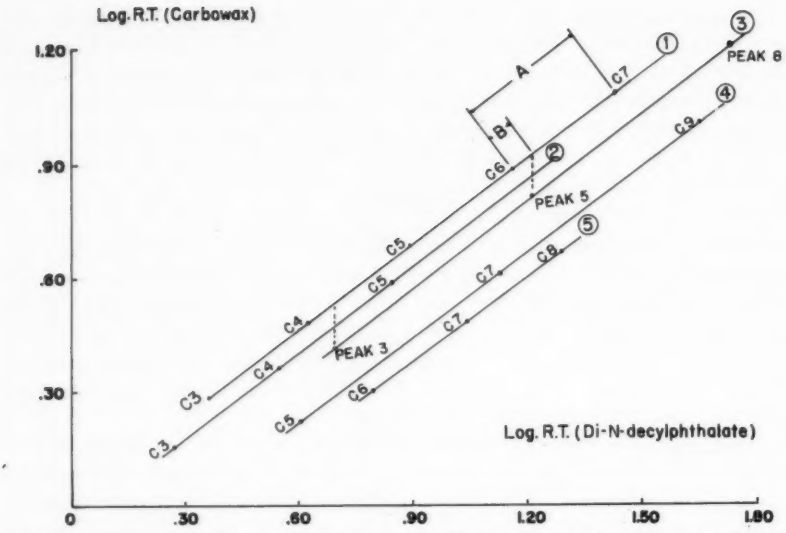

FIG. 2. Log (retention times—min.) in Carbowax-1500 against log (retention times—min.) in di-*N*-decylphthalate. N-amyl alcohol internal standard, T 130° C.—2-meter column lengths. 1. Normal alcohols, three to seven carbon atoms. 2. Iso-alcohols, three to five carbon atoms. 3. Unknowns. 4. Methyl ketones, five, seven, and nine carbon atoms. 5. Ethyl esters, six to eight carbon atoms.

It should be noted that the relationship between boiling points and retention times of components within the same class is not always valid, when forces other than vapor pressure are involved.

To narrow the selection of the most probable alcohol, the number of carbon atoms was established. The 3,5-dinitrobenzoates of the alcohols in an aqueous distillate from Blue cheese were formed following the procedure of Holley *(3)*, and were separated by paper chromatography, using the method of Sundt and Winter *(7)*. Rf values of unknown alcohols when compared to knowns indicated that alcohols with 5, 7, and 9 carbon atoms were present. Using this information and the boiling points derived previously, the unknowns were postulated by consulting boiling-point data *(4)*. The secondary alcohols pentanol-2 (b.p. 119° C.) and heptanol-2 (b.p. 160° C.) came closest to matching the data. These alcohols along with nonanol-2 were then prepared by a sodium reduction of their corresponding ketones—pentanone-2, heptanone-2, and nonanone-2 *(9)*. The retention times of pentanol-2, heptanol-2, and nonanol-2 on two different stationary phases, Carbowax 1500 and di-*N*-decyl phthalate, were identical with Peaks 3, 5, and 8 obtained from Blue cheese. Paper chromatography of their 3,5-dinitrobenzoates also substantiated this conclusion.

Peaks 7 and 9 were not characterized during this study but will be investigated in future work.

Action of mold mycelia on heptanol-2, heptanone-2, and caprylic acid. Pre-

vious investigators have established that *Penicillium roqueforti* is capable of converting fatty acids to methyl ketones having one less carbon atom *(2)*. The mode of formation is postulated to proceed through the beta-oxidation and decarboxylation of the fatty acid *(2, 6)*. In order to investigate the relation of the secondary alcohols found during this study to this scheme, the following experiments were carried out:

A strain of *Penicillium roqueforti* utilized in Blue cheese manufacture,[1] was propagated in a 5.0% malt-extract broth fortified with 2.0% glucose and 0.5% yeast extract. Incubation was carried out for three days at a temperature of 30° C. The medium was constantly agitated during this period to maintain an aerobic state. The mycelia were collected by filtration and washed several times with distilled water. An homogenate of the mycelia was then prepared by mixing thoroughly in a Waring blendor equipped with an homogenate accessory. Phosphate buffer of pH 6.8 *(8)* was used to give a thick slurry of mycelia. Nine milliliters of this slurry plus 30 ml. of phosphate buffer and 0.01-ml. portions of pure caprylic acid, heptanone-2, and heptanol-2 were used in the metabolic experiments. Incubation of the substrates was carried out at 37° C. for 4 hr. At 20-min. intervals during this period the substrates were mixed thoroughly by shaking. Controls on incubated mycelia and buffer also were run.

After incubation, the metabolic products were extracted from the reactants by 20- and 10-ml. portions of di-ethyl ether. The ether extracts were concentrated to 0.5 ml. and analyzed by gas chromatography. The results are presented (Figure 1).

The secondary alcohol heptanol-2 produced the ketone heptanone-2 when incubated with the mold mycelia. Heptanone-2 in turn produced heptanol-2, although the amount formed was approximately one-half the amount of heptanone-2 formed from heptanol-2 after incubation for 4 hr. Caprylic acid plus mycelia produced heptanone-2, but heptanol-2 was not found.

Ripening of Blue cheese. Neutral volatiles from Blue cheese were isolated, using the procedure described previously at different stages during the ripening period and examined by gas chromatography. The secondary alcohols did not appear until considerable amounts of methyl ketones were produced.

DISCUSSION

In light of the previous work by Bassett and Harper *(1)* which indicated that beta-keto acids are present in Blue cheese, it would seem unlikely that the secondary alcohols are a pathway to the methyl ketones. In all probability they result from the reduction of the methyl ketones. The latter is suggested by the following information: *(1)* Methyl ketones appear much sooner and in larger amounts than the secondary alcohols during the ripening of Blue cheese. *(2)* Heptanol-2 was not found during the oxidation of caprylic acid.

The ability of heptanol-2 to form heptanone-2 so rapidly during the metabolic experiments was possibly influenced by the aerobic conditions during the

[1] Hansen Laboratories, Milwaukee, Wisconsin.

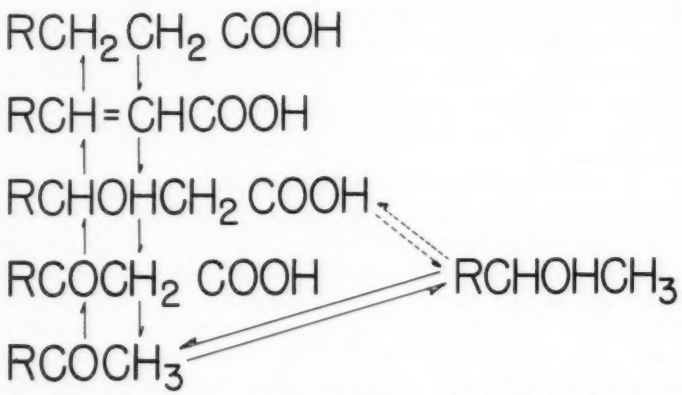

FIG. 3. Suggested metabolic pathway from fatty acids to methyl ketones, and the role of secondary alcohols.

incubation period. A more anaerobic state, of course, would exist within Blue cheese during ripening. Figure 3 illustrates the possible pathway leading to the formation of methyl ketones and the suggested relation of secondary alcohols to this scheme. The possible pathway from the beta hydroxy acid to the secondary alcohol can not be excluded from the data obtained, and until further work has been done it should be presented for consideration.

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VITAMIN CONTENT OF PICKLED CHEESES AS INFLUENCED BY PRODUCTION AND RIPENING [1, 2]

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SUMMARY

The retention of vitamin A, ascorbic acid, thiamine, riboflavin, and niacin was determined during the production of two varieties of pickled cheeses, the Domiati and the Kareish. The effect of subsequent storage in different pickling media under aerobic and anaerobic conditions on vitamin retention also was studied. Vitamin A was retained almost quantitatively in both cheeses during the preparation and also during the anaerobic ripening. However, this vitamin sustained considerable losses during the aerobic ripening. During the preparation of both cheeses, most of the ascorbic acid, thiamine, riboflavin, and the niacin was lost to the whey. The amounts of thiamine and riboflavin retained by the curd decreased during the ripening of the cheese, whereas the amount of niacin increased markedly.

Pickled cheeses constitute an important part of the diet of the lower-income peoples of Egypt since, under their climatic and economic conditions, the making of pickled cheeses has proved practical as a means of utilizing the surplus milk *(7)*. The pickled cheeses are soft in texture and white in color. They may be consumed fresh, but more often they are eaten after ripening in salt solution or pickle. Salting improves the flavor of the cheese and acts as a preservative. The two major varieties of pickled cheeses are Domiati and Kareish. The Domiati cheese is made by rennet coagulation; the Kareish cheese is the product of acid curdling. The Domiati cheese is usually packed in salted milk or whey and stored in tin cans for several months to produce Ripened Domiati cheese. Either Domiati or Kareish cheese may be packed in salted milk or whey which has been inoculated with a culture from a previous batch of cheese, and stored in earthenware pots, usually for no longer than a year, to produce Mish cheeses *(8, 10, 11)*.

Taha and Hamdy *(14)* classified the microorganisms involved in the ripening of Mish cheeses into three groups: lactic acid bacteria; spore-forming aerobes *(Bacillus)*, and spore-forming anaerobes *(Clostridium)*. These authors stated that the function of the lactic acid bacteria and the *Bacillus* group is to develop the proper conditions for the growth of the *Clostridium* group which are the main organisms involved in the ripening of the Mish cheeses.

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[2] Data taken from a thesis presented by Z. I. Sabry to the Graduate School of the Pennsylvania State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[3] Now affiliated with the Faculty of Agricultural Sciences, American University of Beirut, Beirut, Lebanon.

El-Katib *(5)* investigated the nutritive value of Kareish cheese, and demonstrated the influence of the different local practices employed in the preparation of the cheese on its content of fat, protein, and of some vitamins and minerals.

Although extensive research has been carried out concerning factors influencing the vitamin content of other types of cheese *(2, 3, 4, 6, 9, 13)*, there is little information available relating to the nutritive value of pickled cheeses. This investigation was undertaken to study the retention of the milk vitamins during the making of Domiati and Kareish cheeses and to determine the stability of the retained vitamins during the ripening and the storage of the cheeses.

EXPERIMENTAL PROCEDURE

The Domiati cheese was prepared from raw cow's milk, as described by Fahmi and Sharara *(8)*. The cheese was stored in tin cans after being packed in four different types of pickling media: whole milk, skimmilk, commercial buttermilk, and whey, to each of which 5% (by weight) salt had been added. The tin cans were then closed, soldered, and stored at room temperature for 4 and 8 mo., and the ripened product was designated Ripened Domiati cheese. The same types of pickling media were used with the Domiati cheese, which was stored in earthenware pots. Before their use with this cheese, each pickling medium was inoculated with a mother culture,[4] incubated at 35° C. for 48 hr., and then salt was added at the rate of 3% by weight. The pots were closed tightly and stored at room temperature for periods of 3, 6, 9, and 12 mo. to produce Mish Domiati cheese.

The Kareish cheese was made using partly skimmed raw cow's milk following the procedure reported by Saleem *(12)*. When the milk was incubated at 90–110° F., its acidity increased, thereby causing coagulation of milk proteins. The curd was suspended in cheese cloth, in freely circulating air, to allow drainage of whey. It was then cut into small pieces, spread on a wooden board, and fine salt added at the rate of 2 oz. of salt per pound of curd. The salted curd was packed in earthenware pots and the same types of pickling media and storage conditions used with the Mish Domiati cheese were employed in producing the Mish Kareish cheese.

Vitamin A and carotene were determined, using the method described by Henry and Thompson *(11)*, with the exception that the sample was saponified before being extracted with ethyl alcohol, diethyl ether, and petroleum ether. Ascorbic acid was determined photometrically by reduction of 2,6–dichlorophenolindophenol. The thiochrome method was used in analyzing for thiamine. Riboflavin and niacin were determined by microbiological assay. The latter methods have been reported in detail by the Association of Vitamin Chemists *(1)*.

DISCUSSION

The extent of retention of vitamin A, thiamine, riboflavin, and niacin during the production and the ripening of both Domiati and Kareish cheeses is reported (Table 1). At the end of the 4- and the 8-mo. storage periods, the

[4] Received directly from Egypt by air transportation. Apparently, similar to the culture described by Teha and Hamdy.

TABLE 1

Retention of certain vitamins by pickled cheeses during production and ripening

Type of cheese	Vitamin A content when ripened in				Thiamine content when ripened in				Riboflavin content when ripened in				Niacin content when ripened in			
	Whole milk	Skim-milk	Butter-milk	Whey	Whole milk	Skim-milk	Butter-milk	Whey	Whole milk	Skim-milk	Butter-milk	Whey	Whole milk	Skim-milk	Butter-milk	Whey
Ripened Domiati	*(IU/g fat)*[a]				*(μg/g solids)*											
Vitamin in original milk	40.2				3.1				8.5				4.5			
Vitamin in curd	39.3				1.7				6.3				2.2			
Vitamin in fresh cheese	37.8				0.3				4.0				0.5			
Vitamin in cheese at 4 mo.	35.7	35.0	35.4	35.7	0.1	0.1	0.2	0.1	4.2	3.5	3.4	3.0	0.9	0.8	0.7	0.7
Vitamin in cheese at 8 mo.	34.3	34.2	34.4	34.3	0.0	0.0	0.0	0.0	3.7	3.1	3.0	2.5	1.4	1.3	1.0	1.0
Mish Domiati																
Vitamin in original milk	40.2				3.1				8.5				4.5			
Vitamin in curd	39.3				1.7				6.3				2.2			
Vitamin in fresh cheese	37.8				0.3				4.0				0.5			
Vitamin in cheese at 3 mo.	30.5	29.0	32.8	29.8	0.2	0.1	0.1	0.1	3.9	4.6	4.5	4.4	3.6	5.4	9.9	4.7
Vitamin in cheese at 6 mo.	26.0	22.5	28.5	23.9	0.1	0.0	0.0	0.0	3.2	3.9	3.7	3.6	10.0	11.8	15.6	10.3
Vitamin in cheese at 9 mo.	18.4	17.8	24.3	17.8	0.0	0.0	0.0	0.0	1.9	2.1	3.4	2.2	15.8	18.0	28.4	16.0
Vitamin in cheese at 12 mo.	13.3	12.0	22.5	13.9	0.0	0.0	0.0	0.0	1.0	1.5	2.7	1.8	23.5	27.4	41.8	19.9
Mish Kareish																
Vitamin in original milk	40.2				3.0				12.8				5.7			
Vitamin in curd	38.7				2.7				11.8				4.9			
Vitamin in fresh cheese	37.5				0.4				8.6				2.2			
Vitamin in cheese at 3 mo.	33.0	31.2	34.9	32.7	0.2	0.1	0.2	0.1	8.6	8.4	9.0	8.4	10.9	12.4	19.2	11.7
Vitamin in cheese at 6 mo.	29.1	26.6	31.1	27.5	0.1	0.0	0.1	0.0	7.3	7.4	8.8	8.2	22.6	25.9	44.5	24.4
Vitamin in cheese at 9 mo.	22.3	19.9	25.3	22.2	0.0	0.0	0.0	0.0	5.1	4.7	7.7	6.1	38.5	40.7	79.2	39.3
Vitamin in cheese at 12 mo.	19.1	17.1	23.0	17.6	0.0	0.0	0.0	0.0	3.8	3.3	6.9	3.7	56.8	61.7	133.8	57.3

[a] One International Unit (IU) of vitamin A is equivalent to 0.3 μg. vitamin A alcohol, or 0.6 μg. beta-carotene.

TABLE 2

Retention of certain vitamins by ripened Domiati cheese after two periods of aerobic ripening

Ripening period	Percentage niacin retention when ripened in				Percentage riboflavin retention when ripened in				Percentage niacin retention when ripened in			
	Whole milk	Skim-milk	Butter-milk	Whey	Whole milk	Skim-milk	Butter-milk	Whey	Whole milk	Skim-milk	Butter-milk	Whey
(wk.)												
0	100	100	100	100	100	100	100	100	100	100	100	100
2	86	84	83	88	117	123	116	126	174	176	236	130
4	80	75	73	77	130	141	146	133	238	210	388	166

Ripened Domiati cheese was subjected to aerobic conditions for 4 wk. and the average vitamin retention at the end of each 2-wk. interval is reported, percentagewise (Table 2).

Most of the vitamin A (carotene and vitamin A) originally present in milk was retained by the curd of both Domiati and Kareish cheeses. The ripened Domiati cheese, which was stored under anaerobic conditions, retained more vitamin A than did the Mish cheeses. It was observed also that vitamin A was more stable in the Kareish cheese than in the Domiati cheese throughout the production and the ripening processes. This may be attributed to the greater microbiological activity in the Kareish cheese, which may have resulted in a condition less favorable to oxidation.

Ascorbic acid was detected mostly in the whey, whereas the curd of both cheeses was found to be essentially free of this vitamin. Thiamine and niacin and, to a lesser extent riboflavin, were found to be more concentrated in the whey than in the curd of both Domiati and Kareish cheeses. This indicates that part of the riboflavin present in the milk is associated with or attached to the protein. In all instances, the thiamine retained by the curd was rapidly destroyed during the ripening process. The riboflavin content of ripened Domiati cheese and both Mish cheeses decreased during ripening, although to a lesser extent in the cheeses ripened in buttermilk than in those ripened in other pickling

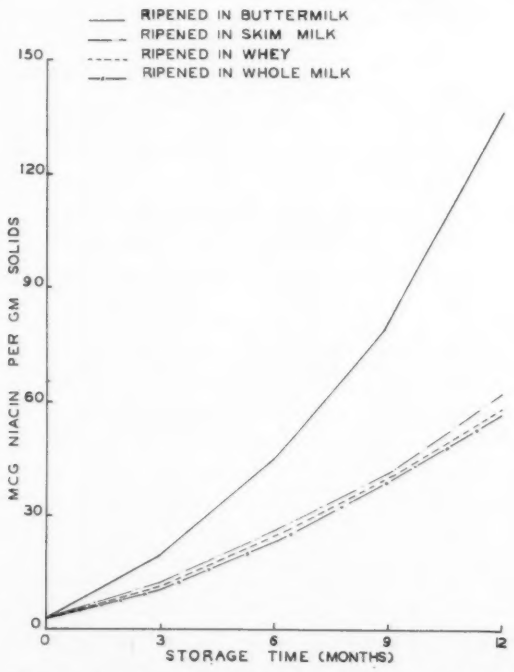

FIG. 1. Effect of ripening of Mish Kareish cheese on the niacin content.

media. This greater retention in the buttermilk medium may be attributed to the higher acidity of the pickling medium. However, the data presented (Table 2) indicate that during the subsequent aerobic ripening of the ripened Domiati cheese, its riboflavin content increased substantially, perhaps due to subsequent alteration of the microbial flora.

The niacin content of both Domiati and Kareish cheeses was found to increase during ripening. However, the strictly anaerobic conditions associated with the ripening of the ripened Domiati cheese proved to be the least conducive to increases in the niacin content. Furthermore, it was observed in all instances that the increase in the niacin content was noticeably higher in the cheeses ripened in buttermilk than in those ripened in other pickling media. Typical data relating to the increase in the niacin content during the ripening of the Mish Kareish cheese are presented (Figure 1). Such an increase in the niacin content could be attributed only to direct synthesis of the vitamin by the ripening microflora. This increase in niacin content is of particular interest, inasmuch as pellagra has been found to be endemic in certain regions of Egypt. This observation should stimulate further studies in this area and attempts should be made to isolate the group or groups of microorganisms responsible for the synthesis of niacin, thereby subsequently improving nutritive value of the pickled cheeses.

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A METHOD FOR MANUFACTURING A HIGH-NITROGEN–LOW-LACTOSE PRODUCT FROM WHEY [1, 2]

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SUMMARY

A method of increasing the total nitrogen of Cheddar cheese whey from a normal content of approximately 0.15 to 0.80% has been devised. The method involves the fermentation of cheese whey with *Lactobacillus bulgaricus*, followed by subsequent treatment of the whey with anhydrous ammonia to neutralize the lactic acid formed during fermentation. Both intermittent and continuous systems of ammoniation were investigated. Concentration of the ammoniated whey was carried to approximately 40, 50, 60, 80, and 90% total solids. Analyses for the concentrated wheys included fat, lactose, ash, and lactic acid. The nitrogen loss during concentration ranged from 4.33 to 8.93% of the total nitrogen present. Nitrogen stability of the concentrated whey stored at room temperature for 153 and 365 days was satisfactory, with a maximum loss of 1.0% nitrogen for 365 days. The concentrated product is syrupy in consistency, salty or acid to the taste, with a color ranging from brown to reddish-brown. Odor descriptions of the concentrated product are silage-like, molasses-like, bouillon-like, and pungent. All concentrations of the ammoniated whey are completely and readily water-soluble. The principal product formed in the process probably is ammonium lactate. Use of nitrogenous bases other than ammonia as lactic acid neutralizers in the process appears feasible; however, this study was limited to an evaluation of ammonia.

Utilization of whey, a by-product of cheese manufacture, is a major problem of the dairy industry. A factor limiting its value for feed, fertilizer, or fermentation usage concerns its low nitrogen content. For example, according to Wegelin *(17)*, the problem of utilizing whey as an animal feed supplement has been hampered by the low protein content of whey in proportion to the salt and lactose content. During the past 10 yr. the nonprotein-nitrogen content of various liquid and solid agricultural products has been increased by treatment with ammonia *(8, 14, 15)*. Products so treated include molasses, citrus solubles, sugar beet pulp, corn silage, and bagasse. The treatment of whey with ammonia affords a means of raising the protein value of the product by increasing the nonprotein-nitrogen content. The work reported herein is a description of a method for ammoniating whey and an outline of the characteristics of the finished product. Some observations on the value of this product as a feed for calves and mature cows will be published.

EXPERIMENTAL PROCEDURE

Manufacture of the Product

The manufacture of ammoniated whey was carried out by two methods. Initially, a manual control system was used, primarily to show that a product

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[1] The material herein reported was taken from the data submitted by the senior author in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Authorized for publication as Paper No. 2227 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

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could be made. Subsequently, an automatic control system was employed, mainly to study the commercial feasibility of the process. The manual control system involved intermittent flow of ammonia into the whey; whereas, the automatic control system involved continuous flow.

Intermittent ammoniation. Batches consisting of 300 gal. of pasteurized Cheddar cheese whey were inoculated with a culture[4] of *Lactobacillus bulgaricus* with mycoderm (a yeast), using a 10% inoculum. The culture was carried in skimmilk prior to addition to the whey. Because of the small volume of each batch of whey and the consequent small amount of culture required, there was no need to produce the culture for use in a step-wise addition as is the custom in industry *(18)*. The whey was incubated at 110° F. until a titratable acidity ranging from 1.00 to 1.50% was attained. The whey then was treated with anhydrous ammonia by allowing the ammonia to pass from the cylinder through a Penberthy injector. The pressure of the ammonia gas caused a mixing of the whey and the ammonia. Ammonia injection was facilitated further by operation of the vat agitator and was continued until the titratable acidity of the fermented whey had fallen approximately to 0.50%. The whey then was allowed to undergo further fermentation and the foregoing procedure was repeated as often as was necessary to reduce the lactose content to 1.0% or lower. Samples of whey were drawn for analyses prior to and following each ammonia treatment. The use of the Penberthy injector was discontinued after the initial three batches of whey were processed. It was noted that proper operation of the injector required that large amounts of ammonia flow into the system. This method of ammonia addition did not permit neutralization of the small amounts of acid produced when processing small volumes of whey. In subsequent operations, a one-half inch steel pipe immersed in the whey was used in place of the injector for adding the ammonia. The anhydrous ammonia used throughout the trials was refrigeration grade, contained in steel cylinders of 150-lb. capacity.

Continuous ammoniation. The continuous ammoniation trials were conducted using a pure culture of *Lactobacillus bulgaricus*. Two levels of inoculation, 5 and 10%, were used in an effort to detect any difference in fermentation. The inoculum was carried in skimmilk as in earlier trials. Fermentation following inoculation proceeded at 110° F. with the continuous ammoniation unit in operation. The conversion of the intermittent ammoniation system to a continuous operation involved the installation of a Foxboro Model 40 Narrow Band Proportional pH Recording Controller, a Beckman Model W Industrial pH Meter, a Foxboro Motor-Driven Needle Valve, and a Beckman Industrial pH Electrode Assembly. The complete assembly is shown (Figure 1). The model W pH Meter is an amplifier designed for measuring the potential generated by the pH electrode immersion assembly. The output signal is used to operate the pH controller which, by means of a control relay, directs the power to a balancing solenoid motor drive which, in turn, controls the air-pressure to open or close the needle valve coupled to the ammonia cylinder. The equipment was operated

[4] Courtesy of the Department of Bacteriology, this institution.

FIG. 1. Assembly for continuous ammoniation process, showing ammonia tank (right), recorder-controller panel (beside tank), electrode assembly (center), and vat with agitator (lower left).

at all times according to the manufacturer's instructions.[5] The instrument was set to maintain a pH of approximately 5.2. This setting was in keeping with the optimum pH for the culture of *L. bulgaricus* supplied by Speck *(13)*.

Samples of whey were taken for analysis at intervals during the trials. The ammonia cylinder was weighed before and after each trial to determine the amount of ammonia used in the process.

Concentration. To achieve a range of total solids with the volumes involved, it was necessary in certain instances to combine batches of product and to employ both an 18-in. (diameter) experimental and a 36-in. commercial Rogers vacuum pan. The whey in all instances was preheated to 160° F., drawn into the pan without holding, and condensed at 25–28 in. of vacuum at temperatures of 140–150° F.

Concentration of the product to a powder was attempted, using a Rogers 50-lb.-per-hr. capacity, single-nozzle spray drier. The ammoniated whey was concentrated to approximately 30% total solids, forewarmed to 180° F., and atomized into the drying chamber at pressures of 1,000, 2,000, and 3,000 lb. Nozzles numbered 66, 72, and 79 were evaluated. Inlet and exhaust air temperatures were approximately 275 and 180° F., respectively, for all drying trials.

[5] Complete details of construction and operation of the equipment are available in Foxboro Bulletins 11-360, 11-361, 11-400, 12-215, 12-221, and 16-350. The Foxboro Company, Foxboro, Mass. Also, in Beckman Bulletin 343-B, Beckman Instruments, Incorporated, Fullerton, California.

Examination of the Product

Whey used in the trials was examined both before and after ammoniation and after concentration. The very viscous samples of higher total solids content were warmed to 50° C. and mixed by stirring with a spatula. Duplicate analyses were performed on all samples and repeated if the results were not comparable.

Lactose content of the whey was determined by the picric acid method of Perry and Doan *(11)*. A standard curve was derived for lactose. A Klett-Summerson photoelectric colorimeter employing a No. 52 filter (520 mμ) was used for all color comparisons. Nitrogen determinations were made according to the Kjeldahl method described by Triebold *(16)*. Copper sulfate was used as a catalyst and the ammonia was collected in saturated boric acid. Ammonia nitrogen was determined by the method of Perkins, Merrill, and Rogers *(10)*. Analyses for lactic acid were made by the method of Barker and Summerson *(3)*, as modified for dairy products by Davidson *(6)*. The method involves the conversion of lactic acid to acetaldehyde by treatment with concentrated sulfuric acid and the determination of acetaldehyde by its color reaction with *p*-hydroxydiphenyl in the presence of cupric ions. A Klett-Summerson colorimeter was used for color comparisons with a No. 56 filter (560 mμ). A standard curve for lactic acid was prepared. The ash content of the concentrated whey was determined by the A.O.A.C. method *(2)* for dairy products. The fat content of the concentrated ammoniated whey was determined by the solvent extraction method of Mojonnier and Troy *(9)*. Moisture analyses were made according to the A.O.A.C. method of drying to constant weight over sulfuric acid *(2)*. The nitrogen stability of the concentrated whey was determined by storage of the product at room temperature in 500-ml. glass containers with plastic screw-cap closures, and subsequent analyses for nitrogen at varying intervals of time. Total solids were determined at the beginning and end of the storage period.

RESULTS AND DISCUSSION

Intermittent ammoniation. The experimental work using manual operation of the ammoniating system was conducted to determine if the nitrogen content of the whey could be increased. An analysis of the data (Figures 2 and 3) shows to what extent this was achieved in eight trials. Figure 2 concerns change in nitrogen content and Figure 3 presents data on change in lactose content for these trials. In general, it was found possible to increase the nitrogen content of the whey from approximately 0.15%, which is within its normal range *(1, 4)*, to the vicinity of 0.8%, and to decrease the lactose content from 5 to about 1%.

The lengthy fermentation periods of 70 hr. or more noted in the first three trials might have resulted from the culture used. Campbell's *(5)* statement that a culture of *L. bulgaricus* without mycoderm gives good production of lactic acid led to the utilization of such a culture. The lower portions of Figures 2 and 3 represent the data collected from Trials 4 through 8 in which *L. bulgaricus* alone was used. Although the decrease in lactose and increase in nitrogen content in these trials was slightly improved from the viewpoint of fermentation time over

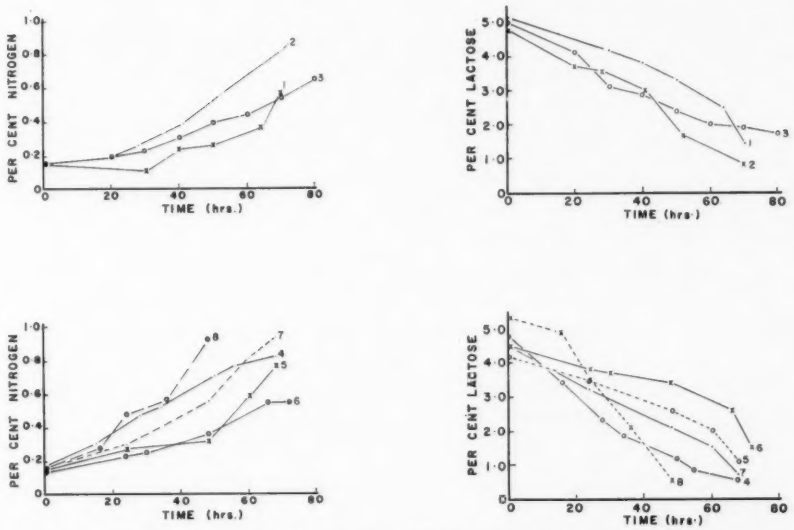

FIG. 2. Change in nitrogen content of liquid whey, using 10% inoculum and intermittent ammoniation. Upper—starter containing *L. bulgaricus* and mycoderm. Lower—starter without mycoderm.

FIG. 3. Change in lactose content in liquid whey, using 10% inoculum and intermittent ammoniation. Upper—starter containing *L. bulgaricus* and mycoderm. Lower—starter without mycoderm.

that noted for *L. bulgaricus* with mycoderm, it was still considered to be too long for practical commercial operation.

In the foregoing trials, conducted by manual addition of ammonia, 10% represented a practical upper limit to the amount of starter addition. Thus, some other means of reducing fermentation time was sought. Automatic control of the ammonia addition to maintain a constant optimum pH was felt to be a logical way to overcome this difficulty.

Continuous ammoniation. The apparatus for continuous automatic addition of ammonia described previously was observed to feed the anhydrous ammonia at a steady rate which maintained the pH of the whey at a constant level of 5.2. Analyses made at various times throughout the fermentation period indicated that the problem of lengthy fermentation periods could be overcome with this instrumentation. The data (Figures 4 and 5) show that continuous ammoniation enables increase in the nitrogen content of the whey to 0.6% or higher in less than 14 hr. when 10% inoculum is used, and in less than 17 hr. with 5% inoculum. The decrease in lactose closely follows the pattern of nitrogen increase, as can be seen (Figure 6).

The data of Trials A2, A8, and A10 are not included in Figures 4 and 5, as these trials, all at 5% inoculum, were observed to be undergoing a putrefactive type of fermentation. The complete failure of these three trials is difficult to

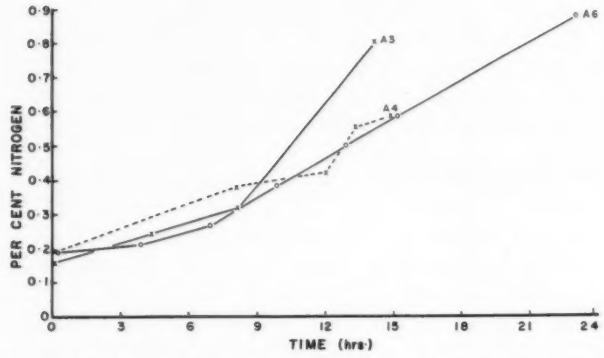

FIG. 4. Change in nitrogen content in liquid whey, using *L. bulgaricus* starter (5% inoculum) and continuous ammoniation.

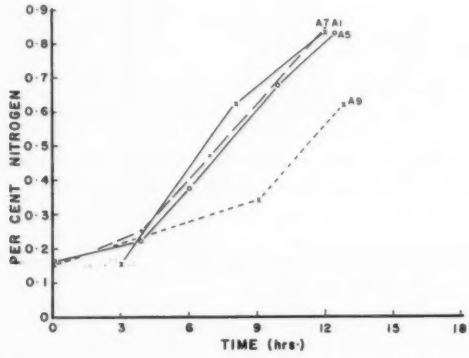

FIG. 5. Change in nitrogen content in liquid whey, using *L. bulgaricus* starter (10% inoculum) and continuous ammoniation.

explain, since all the whey was pasteurized. Antibiotic contamination from the original milk is a distinct possibility, because antibiotic detection tests have indicated such contamination from time to time in the cheese milk received at the University Creamery. No bacteriophage failures in the commercial cheese operations were recorded during the period of these experiments. Precise explanation for batch failures can not be offered at this time but it would appear that higher levels of inoculum afford a margin of safety.

Probable reaction involved. The chemical reaction responsible for the nitrogen increase in the whey was not thoroughly investigated. Necessarily it would involve an acid-base reaction resulting in a salt. The salt should be ammonium lactate. This would mean that the production of lactic acid from lactose by fermentation, followed by ammoniation, would involve a relationship between lactose decrease and nitrogen increase in the whey. This relationship is confirmed (Figure 7). Using the method of Snedecor *(12)*, it is shown that the

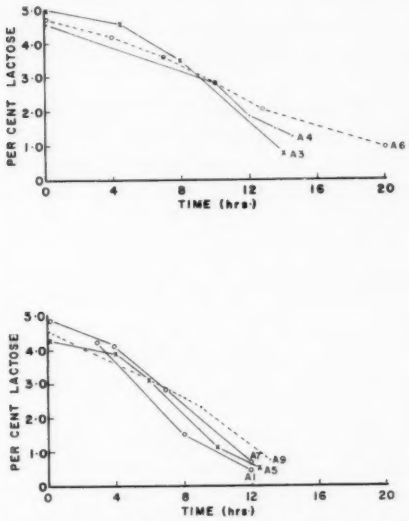

FIG. 6. Change in lactose content in liquid whey, using *L. bulgaricus* starter and continuous ammoniation. Upper—5% and lower—10% inoculum.

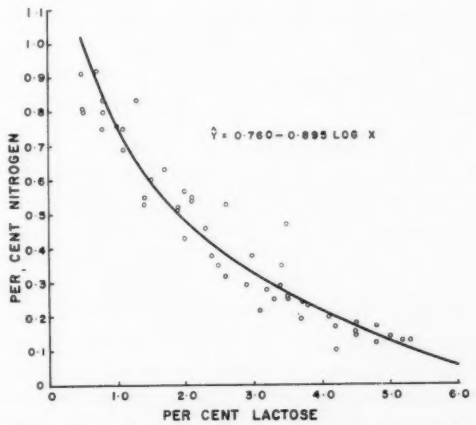

FIG. 7. Relationship between the amounts of lactose and nitrogen during ammoniation of liquid cheese whey.

nitrogen uptake can be predicted from the lactose content of the whey with a Standard Error of $\pm$ 0.024. Although this is not offered as proof that ammonium lactate is the product formed, it is offered as evidence that a reaction occurs between the lactic acid and the anhydrous ammonia added.

Amount of anhydrous ammonia required for ammoniation. The amount of ammonia required to ammoniate the liquid cheese whey was determined from

batches prepared with the automatic system, as this was concluded to be more applicable to industrial use. It was calculated from mean values that 0.012 lb. of ammonia was required to increase the per cent nitrogen of a gallon of whey by 0.10%.

Concentration. Concentrated products having five levels of total solids (Table 1) were produced. The ammoniated whey was observed to condense well in the vacuum pan at levels of vacuum and temperature used for skimmilk. Foaming was not a problem and any residue in the pan was very water-soluble and thus easy to rinse off.

TABLE 1

Approximate composition of concentrated ammoniated whey

	Sample				
	1	2	3	4	5
	(%)				
Total nitrogen	4.60	5.31	6.33	8.00	9.02
Total nitrogen, dry basis	10.55	10.47	10.22	10.17	10.10
Ammonia nitrogen	3.65	4.16	5.03	6.60	7.39
Ammonia nitrogen, dry basis	8.37	8.20	8.12	8.39	8.27
Ammonia nitrogen as % of total nitrogen	79.35	78.34	79.46	82.50	81.93
Total solids[a]	43.59	50.73	61.94	78.69	89.30
Protein[b]	6.10	7.34	8.29	8.93	10.40
Ammonia	4.74	5.41	6.54	8.58	9.61
Fat	0.64	0.60	1.00	1.28	1.61
Ash	3.26	3.81	4.60	6.05	6.54
Lactose	2.96	6.31	5.66	3.80	8.92
Lactic Acid	20.06	24.75	30.41	.....	44.59
Total solids not accounted for	5.83	3.51	5.44	.....	7.63
pH	5.3	5.5	5.7	5.7	5.8

[a] Determined by A.O.A.C. drying-without-heat method *(2)*.
[b] A protein factor of 6.38 was used as the value accepted for dairy products.

Nitrogen loss during concentration was calculated from trials, using the 18-in. vacuum pan. The loss in nitrogen varied from slightly over 4.0 to almost 9.0%. The highest loss was recorded with the highest level of concentration, although the relationship did not hold true in all cases. The 18-in. vacuum pan used in the trials was not equipped with an entrainment separator and according to Hunziker *(7)*, as much as 2.0% of the product in the batch can be lost without such a separator. Thus, the losses recorded were not considered great enough to be a major problem in a commercial operation utilizing adequate protection against entrainment loss. Of course, loss of ammonia per se would not be prevented by an entrainment separator. Although not evaluated in the present study, saturation of the concentrated product with additional ammonia affords an obvious means both of overcoming ammonia losses and of achieving maximum level of ammoniation.

The concentrated ammoniated whey varied from a liquid with viscosity of heavy cream to a thick viscous syrup, depending on the level of concentration. All levels of concentration were readily and completely water-soluble. The color of the product varied from a caramel-brown at the lower levels of concentration to a dark brownish–red color at extreme concentrations. The odor of the concentrated product was not unpleasant, and was described by various observers as

silage-like, molasses-like, bouillon-like, or pungent. The product was unpleasant to taste, being salty to some observers and strongly acid to others.

Attempts to dry the concentrated ammoniated whey resulted in a sticky product, which coated the inside of the drier and the drier collecting-bags. This product remained syrupy without any evidence of hardening. Addition of a suitable solid carrier to the concentrated whey might have facilitated drying of the product. However, this possibility was not evaluated.

Approximate compositions of five concentrated products, made from various combined batches of ammoniated whey, are shown (Table 1). It was recognized early in the investigation that analysis of moisture in the product would be difficult due to the possibility that the salt formed during the ammonia treatment would decompose readily into a volatile constituent (ammonia) with heat. Therefore, the A.O.A.C. method of drying without heat over sulfuric acid was selected as the best moisture method available.

The data of Table 1 show that a portion of the total solids of each batch is not accounted for. The limitations of the moisture test and the possible error of the lactic acid determination, as discussed by Davidson *(6)*, may have contributed to this difference. In addition, the process involves a fermentation procedure which could give rise to compounds not detectable by the analytical techniques used. Considering these limitations some unaccounted solid material might be expected.

It is noteworthy that (Table 1) the total nitrogen and total ammonia nitrogen calculated on a dry basis for each concentration level are of the same magnitude. This indicates that in each case the maximum level of nitrogen that can be achieved by the process is 0.8 to 0.9% in the liquid whey.

Keeping quality. As the commercial use of a concentrated ammoniated product would necessarily involve storage, stability of the added nitrogen is of importance. The data (Table 2) refer to the storage trials described earlier. All the samples stored well, from the viewpoint of odor and visual examination. Sample 3, stored for 365 days, showed signs of liquefaction, indicated by loss of viscosity, but did not deteriorate as far as could be discerned by odor or appearance. The change in nitrogen content after 153 days of storage was not regarded as significant, perhaps being due to errors in sampling. The slight

TABLE 2

Nitrogen stability in concentrated ammoniated whey stored at room temperature

	0 Days sample			153 Days sample		365 Days sample
	1	2	3	1	2	3
Total solids	89.30	61.94	78.69	90.64	63.70	82.07
Total nitrogen	9.02	6.33	8.00	9.32	6.51	7.60
total nitrogen, dry basis	10.10	10.22	10.17	10.28	10.22	9.26
Per cent change in total nitrogen, dry basis				+ 0.18	0.00	− 0.91
Ammonia nitrogen	7.39	6.03	6.60	7.30	5.14	6.00
Ammonia nitrogen, dry basis	8.28	8.12	8.39	8.05	8.07	7.31
Per cent change ammonia nitrogen, dry basis				− 0.23	− 0.05	− 1.08

loss of nitrogen in Sample 3 on storage for 365 days appears to be accounted for by loss in ammonia nitrogen. The results presented (Table 2) also show a loss in moisture content for all samples, as would be expected in containers that were not air-tight. Considering the relatively low levels of nitrogen lost, even after 365 days, it can be assumed that the nitrogen stability of the concentrated ammoniated whey stored at room temperature is adequate for commercial practices.

CONCLUSIONS

Results of this investigation have shown that the treatment of cheese whey undergoing a lactic acid–type fermentation, with anhydrous ammonia, is a commercially feasible process for producing a high-nitrogen whey product. This product can be concentrated without difficulty to a total solids level of 90% and might be used as a feed for ruminants, as a fertilizer, or as a fermentation additive. The process has shown that the lactose of whey can be completely fermented in 12–14 hr. when anhydrous ammonia is used as neutralizer. This fermentation period is much shorter than the times reported in the literature concerning commercial lactic acid production, when lime is used as a neutralizer. It is, therefore, noted that a method for the production of lactic acid might be devised, using anhydrous ammonia as a neutralizer.

ACKNOWLEDGMENT

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MODE OF ACTION OF HYPOCHLORITE SANITIZERS WITH AND WITHOUT SODIUM BROMIDE [1]

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SUMMARY

Increases in bactericidal effectiveness of solutions of a hypochlorite sanitizer of high alkalinity with $NaBr \cdot 2H_2O$ added were related directly to the concentrations of $NaBr \cdot 2H_2O$, with the effect of chlorine being negligible. Solutions containing constant concentrations of $NaBr \cdot 2H_2O$ and increasing concentrations of the alkaline hypochlorite sanitizer decreased in relative effectiveness with increasing concentrations of the sanitizer. This reduction in effectiveness appeared to be related to increases in pH of the solutions. The term active chlorine is proposed to express the per cent of the total available chlorine obtained by titration of a chlorine solution without acidification. The amount of active chlorine in a hypochlorite solution is related to the pH of the solution; consequently, to the bactericidal effectiveness and corrosiveness of the solution. Explanation is offered as to the reason why $NaBr \cdot 2H_2O$ increases the effectiveness of hypochlorite solutions of high alkalinity. The merits of considering bacterial resistance to germicides on the basis of species, rather than on the basis of the gram-stain reaction, are discussed.

Additions of small amounts of sodium bromide improve the bactericidal effectiveness of solutions of an alkaline hypochlorite sanitizer, as previously reported *(6)*. A possible explanation for the improvement may be the formation of hypobromous acid due to a chemical reaction between chlorine and sodium bromide and the greater bactericidal effectiveness of hypobromous acid compared to hypochlorous acid at the pH of the solution.

The sanitizer and the $NaBr \cdot 2H_2O$ produce colorless solutions when dissolved individually in water. However, when the two are mixed together and dissolved, a yellow-colored solution results. The intensity of the yellow color is directly related to the concentration of $NaBr \cdot 2H_2O$ as measured on a Beckman Spectrophotometer Model DU, wave length 400 mμ.

Due to greater reactiveness of chlorine over bromine, the following reaction takes place when NaBr is added to a chlorine solution:

$$Cl_2 + 2NaBr \longrightarrow Br_2 + 2NaCl$$

At pH 11, the approximate pH of the system under study, bromine will be present partly as NaOBr and partly as OBr^-; hence, the yellow color of the solutions.

Bromine, like chlorine, is a powerful germicide. The compounds are closely related chemically. The state at which they are present in solution determines greatly the bactericidal effectiveness of each. The following equations, based on data by Holst *(2)*, Wyss and Stockton *(10)*, and Marks and Strandskov *(7)* illustrate the approximate state of the two elements at various pH levels:

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$$\underset{\text{pH 1}}{Cl_2} \underset{\text{pH ?}}{\rightleftharpoons} \underset{\text{pH 4–5.8}}{HOCl} \underset{\text{pH 7.2–7.4}}{\rightleftharpoons} \underset{\text{pH 9.3}}{ClO^-}$$

$$\underset{\text{pH 3}}{Br_2} \underset{\text{pH 5}}{\rightleftharpoons} \underset{\text{pH 6–8}}{HOBr} \underset{\text{pH 9}}{\rightleftharpoons} \underset{\text{pH 10.5}}{BrO^-}$$

The pH values under the formulae are those at which the specified compounds are predominant, and those under the arrows are the approximate pH values at equilibrium. The equations illustrate that the states of bromine which correspond to those of chlorine exist at about two units higher on the pH scale. For example, chlorine is present at HOCl at pH 4–5.8, whereas the corresponding state for bromine, HOBr, is at pH 6–8.

Marks and Strandskov *(7)*, using spores of *Bacillus metiens*, demonstrated that chlorine was more effective than bromine below pH 8. At pH above 8, the effectiveness of both germicides decreased. However, the decrease in the effectiveness of chlorine was much greater than in bromine effectiveness, a result which may be related to the state of the two compounds at various pH levels.

The release of bromine from $NaBr \cdot 2H_2O$ by part of the chlorine, and the greater germicidal effectiveness of bromine over chlorine at high pH apparently, then, is the reason for the increase in germicidal effectiveness of mixtures of $NaBr \cdot 2H_2O$ and the alkaline hypochlorite sanitizer over that of the sanitizer alone.

The present paper deals with *(a)* the effect of the concentration of sodium bromide and pH on the bactericidal effectiveness, *(b)* observations on the effectiveness of hypochlorite sanitizers, and *(c)* the resistance of gram-positive and gram-negative bacteria to chemical sanitizers.

EXPERIMENTAL PROCEDURE

The sanitizer and the techniques used for determining bactericidal effectiveness were those listed in the previous report *(6)*. Sodium bromide was used as crystalline $NaBr \cdot 2H_2O$.

Chlorine, as well as bromine, was determined quantitatively by the conventional titration procedure, using KI and $Na_2S_2O_3$.

Since the reaction between chlorine and bromine is on a molar basis, the apparent strength of a chlorine solution will not change if bromine is substituted for chlorine in a solution of given strength. For simplicity, the strength of a solution is always referred to as p.p.m. of available chlorine, rather than listing the specific contents of chlorine and bromine.

Effect of the concentration of $NaBr \cdot 2H_2O$ and pH. The results reported in the previous article *(6)* indicated that the bactericidal effectiveness of the sanitizer increased with increasing concentrations of $NaBr \cdot 2H_2O$. It also was apparent that the relative effectiveness of specific increments of $NaBr \cdot 2H_2O$ was greater at low than at high concentrations of the sanitizer.

Additional studies were made of the bactericidal effectiveness of solutions containing various concentrations of $NaBr \cdot 2H_2O$ and constant levels of available chlorine (20 p.p.m.). The test bacterium was *Micrococcus* species

MS-102, an organism which is comparatively resistant to hypochlorite. The systems had identical pH, of 10.8. The results are illustrated in Figure 1, where the concentrations of $NaBr \cdot 2H_2O$ are plotted against the logarithm of the time required for 99.999% destruction of the bacteria.

The germicidal effect of a mixture of chlorine and bromine would be due to the combined effect of both. However, it appears that under the conditions of this experiment, the effectiveness of a mixture perhaps is related directly to the concentration of bromine, and that the effect of the chlorine is negligible. Assuming complete reaction between hypochlorite and bromide, the molar ratios of $Cl_2 : Br_2$ in the mixtures varied from 8.1 : 1 to 1.2 :1, and the effectiveness of the mixture increased approximately ten times from the lowest to the highest concentration of $NaBr \cdot 2H_2O$. From the slope of the curve, it appears that

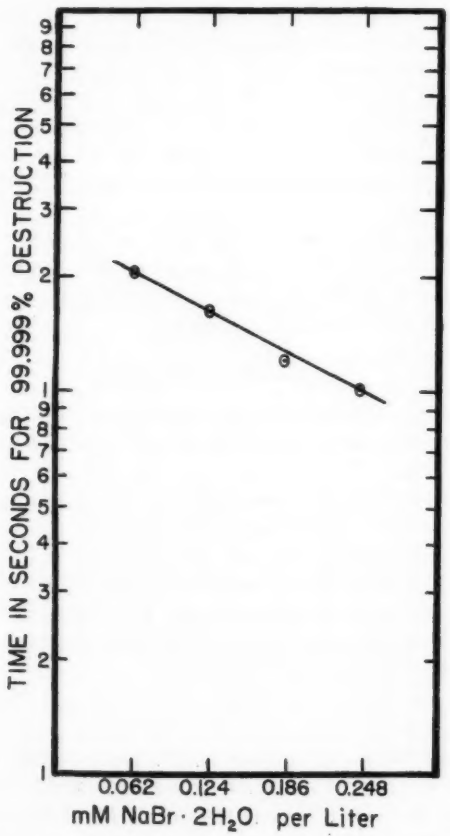

FIG. 1. The bactericidal effectiveness of a sanitizer solution containing 20 p.p.m. of available chlorine and varying concentrations of $NaBr \cdot 2H_2O$ at pH 10.8. Test organism: *Micrococcus* species MS-102.

bromine may be about 100 times more effective than chlorine at pH 10.8, in pure solutions of equimolar concentration.

The effect of varying the concentrations of the hypochlorite sanitizer, while keeping the NaBr · $2H_2O$ concentration constant, was demonstrated in the following experiment. In this series, the chlorine content ranged from 5–70 p.p.m. and the concentration of NaBr · $2H_2O$ was 0.09 m*M*/liter of solution. The molar ratios of $Cl_2 : Br_2$ varied from 0.6 : 1 to 21 : 1. The test bacterium was *Microbacterium lacticum* H2. The results are plotted (Figure 2) as the logarithm of the number of survivors after 30 sec. of exposure at increasing concentrations of chlorine.

The results illustrate that mixtures of the sanitizer and NaBr · $2H_2O$ become less effective with increasing amounts of hypochlorite. This may be due to an inhibitory effect of the chlorine on the bromine. However, pH also must be considered. At 10 p.p.m. of hypochlorite, the pH was 10.4, whereas between 20 and 70 p.p.m. of hydrochlorite, the pH ranged from 10.8 to 11.0. Apparently, even a few tenths of a pH unit greatly alters the effectiveness of the mixture.

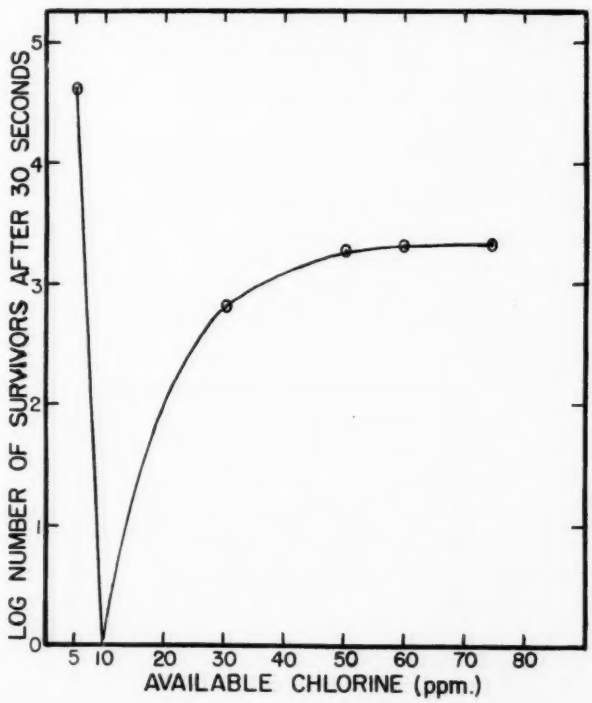

FIG. 2. The bactericidal effectiveness of a sanitizer solution containing varying concentrations of available chlorine and a constant concentration of NaBr · H_2O. Test organism *M. lacticum* H-2.

At the higher concentrations of chlorine, where the pH is relatively constant, larger amounts of chlorine did not increase the effectiveness of the mixture. This observation confirms the results of the previous experiment in that the concentration of bromine rather than the concentration of chlorine, determined the effectiveness of the mixture.

The decrease in effectiveness of the mixture below 10 p.p.m. of hypochlorite, even though the pH and the ratio of $Cl_2 : Br_2$ of the system were more favorable for faster action than at higher concentrations of hypochlorite, probably was owing to a below-minimum concentration of oxidative agents for the amount of organic material present.

The results illustrated (Figures 1 and 2) agree well with those obtained by Marks and Strandskov *(7)*, who studied the germicidal effect of both chlorine and bromine on spores of *B. metiens*. Their results and the slope of their curve would indicate that bromine is about four to five times as effective as chlorine against spores of this bacterium at pH 9, and that the difference would become even greater at increased pH levels.

When mixtures of the sanitizer and $NaBr \cdot 2H_2O$ were compared to the sanitizer alone in the presence of 200 p.p.m. of temporary hard water, the differences in germicidal effectiveness were negligible *(6)*. The pH of this system was approximately pH 8, thus confirming the results of Marks and Strandskov *(7)*, which showed that chlorine and bromine become equally effective at pH 8.

Effectiveness of hypochlorite sanitizers. When the available chlorine content of a solution of hypochlorite is determined by titration with $Na_2S_2O_3$, the solution is first acidified in order to decrease pH to a level at which iodine is liberated quantitatively from KI as iodine. The pH of titration is about pH 2–3, well below the pH at which the solution is actually used for sanitization. For food plants to obtain the best protection from their sanitization program, it may be well for users of chlorine compounds to consider not only the concentration of available chlorine but also how active this chlorine is under the conditions of sanitization.

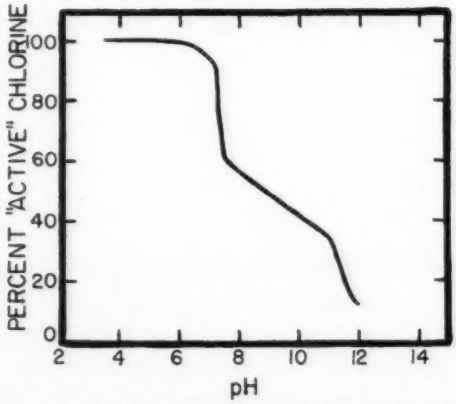

FIG. 3. The per cent of active chlorine in a hypochlorite solution at various pH levels.

Results obtained by titration of a hypochlorite solution containing 100 p.p.m. of available chlorine at pH levels from 4.0 to 12.0 are illustrated (Figure 3). As the pH of the solution was increased, less $Na_2S_2O_3$ solution was needed for titration. This results because the state to which $Na_2S_2O_3$ is oxidized by iodine is dependent on pH. In normal chlorine determination (pH 2 to 3), thiosulfate is oxidized to tetrathionate and one mole of thiosulfate corresponds to one equivalent of iodine. If the solution is not acidified, the thiosulfate is oxidized in part or completely to sulfate, depending on the pH of the solution. The oxidation of thiosulfate to sulfate requires eight equivalents of iodine per mole of sulfate *(4)*.

The results (Figure 3) are recorded as active chlorine, which is the ratio times 100 of the titer of a chlorine solution at any given pH to the titer of a chlorine solution of similar strength at pH of normal titration. The strong resemblance between this curve and the dissociation curve for hypochlorous acid indicates that the $Na_2S_2O_3$ titer of iodine at any given pH is closely related to the state of chlorine at that pH; therefore, to the germicidal effectiveness of chlorine.

The effectiveness of a hypochlorite solution is directly related to the content of undissociated hypochlorous acid *(8)*. The chlorine is negligible and does not become a factor until pH is lowered to about one, where about 15% of the chlorine is present in the molecular form *(1)*. The content of un-ionized hypochlorous acid is at a maximum at a pH of about 5.8. As pH is increased above 5.8, hydrochlorous acid becomes increasingly more dissociated; thereby, increasingly more ineffective. Complete dissociation occurs at about pH 9.3.

Although hypochlorite solutions have maximum germicidal effectiveness at pH 6 and below, solutions of this low pH are never used in practice, as hypochlorites also have maximum instability and corrosiveness at this pH. Solutions of commercial hypochlorite sanitizers range in pH from 8–11 at 100 p.p.m. of available chlorine. As can be seen (Figure 3), the titration curve begins to flatten out as pH becomes greater than pK (pH about 7.4), and remains comparatively flat until at about pH 11, where it again becomes rather steep.

The pH region 7.4–11, within which commercial hypochlorite sanitizing solutions fall, was demonstrated to be the region in which maximum stability and minimum corrosiveness are combined with germicidal efficiency. The lower the pH of a solution in this region, the more effective and the more corrosive the solution becomes. These trends are reversed as pH increases. Below pH 7.4, hypochlorite solutions become too unstable and corrosive for practical use, and above pH 11 they become almost germicidally ineffective. The state in which chlorine exists in this pH range is about evenly divided between HOCl, ClO^-, and ClO^-, the dividing point being about pH 9.3.

The proposed new term—active chlorine—may be of practical value. The active chlorine would be the part of the total available chlorine which is active under the prevailing conditions of sanitization. By performing chlorine titration without the addition of acid, in addition to the regular titration for available

chlorine, the per cent of the available chlorine present in active form can be calculated and sanitization procedures regulated accordingly.

The percentages of chlorine in the active state in commercial hypochlorite-type sanitizers range from 30–48%. Circulation for 10 min. of any hypochlorite solution containing approximately 100 p.p.m. of available chlorine, as recommended by most manufacturers, will result in satisfactory sanitization. However, solutions containing 40–48% active chlorine should not be left in contact with equipment over excessively long periods of time, if corrosion is to be minimized. With solutions containing less than 40% active chlorine, the danger of corrosion is not as great as with solutions containing more than 40% active chlorine.

The differences in the percentage of active chlorine between corrosive and noncorrosive solutions may appear small. However, it should be remembered that in solutions containing over 40% active chlorine, part of the chlorine is present as HOCl. This makes such solutions comparatively much more corrosive than solutions containing less than 40% active chlorine, where the chlorine is present as ClO^-.

The hardness of the water will affect the pH of a hypochlorite solution; consequently, the amount of chlorine present in the active state. For this reason, determination of active chlorine will need to be made in each plant, in order to establish the corrosion potential of the use-solution under the existing conditions.

Resistance of gram-positive and gram-negative bacteria to chemical sanitizers. Hypochlorite is considered relatively more effective against gram-negative bacteria than against gram-positive; whereas, the reverse is considered to be the case for quaternary ammonium compounds. However, a great variation exists in the resistance of gram-positive and gram-negative bacteria to hypochlorite, as is evident from the results in previous reports *(5, 6)*. Some gram-negative bacteria were more resistant than some gram-positive bacteria to hypochlorite sanitizers of relatively low alkalinity. Similar relative resistances were indicated using quaternary ammonium compounds. For example, *Pseudomonas fluoroscens* (gram-negative) was more resistant to both types of sanitizers than was *Microbacterium lacticum* H2 (gram-positive).

When evaluating the relative effectiveness of sanitizers against various bacteria, perhaps the mode of action of the sanitizer should be considered. The effectiveness of quaternary compounds is considered to be owing to the inhibition of certain nonspecified enzymatic processes. The mode of action of hypochlorite is threefold: *(a)* the irreversible reaction of chlorine with enzymes containing −SH groups *(3)*, *(b)* the reaction of chlorine with amino acids and peptides *(9)*, and *(c)* the oxidative properties of hypochlorous acid *(1)*.

Although no actual data are presented, it is often evident in morphological examinations that gram-positive bacteria contain more mass than do gram-negative bacteria. Gram-positive bacteria such as certain micrococci are often capsulated and, usually, the greater the capsulation the more difficult it is to destroy the bacterium. Perhaps the reason hypochlorite, which acts both as an inhibitor and an oxidizing agent, is relatively less active against certain gram-positive bacteria than against others, could be that a certain amount of the

chlorine is utilized by the organic nonvital capsule material; thus, is unavailable for acting on the bacterial enzyme system. In case of gram-negative or non-capsulated bacteria, the bacterial enzyme can be acted upon almost instantaneously by the hypochlorite.

The rapidity with which quaternary compounds act may be dependent only on the speed of penetration of the cell wall and/or capsule of the bacterium. If the organism is capsulated, the sanitizer requires a longer time to penetrate, but little if any of the sanitizer is lost due to combination with other components of the system, as in the case of chlorine.

Although the matter is of little practical significance, it may be well to consider the resistance of bacteria to chemical sanitizers on a species basis, rather than on the basis of staining-ability characteristics. The latter may be misleading, because these characteristics per se probably are only incidental to resistance.

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EFFECT OF SODIUM BROMIDE ON THE BACTERICIDAL EFFECTIVENESS OF HYPOCHLORITE SANITIZERS OF HIGH ALKALINITY [1]

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SUMMARY

Studies were conducted, in which comparisons were made of a standard hypochlorite sanitizer of high alkalinity (pH 11 at 100 p.p.m.) and the same sanitizer to which was added crystalline $NaBr \cdot 2H_2O$. Addition of the $NaBr \cdot 2H_2O$ improved the germicidal effectiveness of the hypochlorite sanitizer under laboratory conditions. The rate of increase in effectiveness varied with the concentration of $NaBr \cdot 2H_2O$ and the test organism. A standard mixture of 1.35% $NaBr \cdot 2H_2O$ and the sanitizer resulted in a 33 to 1,000% increase in bactericidal effectiveness against a variety of bacteria, in comparison to results obtained by using the sanitizer alone. Mixtures of 4% of $NaBr \cdot 2H_2O$ and the alkaline sanitizer compared favorably in germicidal effectiveness against all test organisms to a hypochlorite sanitizer of relatively low alkalinity.

Variations in temperature and addition of 0.5% skimmilk and hard water affected the bromide-hypochlorite mixture in ways similar to those in which other hypochlorite-type sanitizers were affected. However, under these conditions, the mixture was usually superior in germicidal effectiveness to the alkaline sanitizer alone. The corrosiveness of the sanitizer was not affected by addition of 1.35% $NaBr \cdot 2H_2O$.

Hypochlorite-type sanitizers, supplied either in liquid or in powdered form, have been accepted generally by all phases of the food industries. These sanitizers are composed of the active ingredient, hypochlorous acid, mixed with inert ingredients. Hypochlorous acid is present either as the calcium or as the sodium salt. The common inert ingredients include calcium hydroxide, sodium carbonate, and tricalcium phosphate.

The chemical nature of the inert ingredient has a direct effect on the germicidal effectiveness and corrosiveness of a hypochlorite-type sanitizer, by establishing the pH of the solution in which the sanitizer is placed. Solutions of commercial hypochlorite-type sanitizers vary in pH from approximately 8 to 11 in concentrations of 100 p.p.m. available chlorine, with a pH of approximately 8 being the most common.

An ideal dairy sanitizer is one of high germicidal effectiveness, high stability, and no corrosiveness. Hypochlorite-type sanitizers of high alkalinity possess two of these characteristics: high stability and minimum corrosiveness. However, when tested under laboratory conditions, the high pH sanitizers are somewhat less effective than hypochlorites of low pH *(4)*. This difference becomes of minor significance under practical conditions.

An effort to improve the germicidal effectiveness of hypochlorite-type sanitizers of high alkalinity led to the discovery that additions of small amounts

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either of the potassium or of the sodium salts of hydrobromic acid had a beneficial effect *(2)*. The salts by themselves were bactericidally ineffective. These observations resulted in additional investigation, which is the basis for this paper.

EXPERIMENTAL PROCEDURE

A commercial hypochlorite-type sanitizer of high alkalinity was used to study the effect of addition of sodium bromide. Solutions of this sanitizer containing 100 p.p.m. of available chlorine had a pH of 11. Addition of sodium bromide did not alter the pH of the solutions. Sodium bromide was added directly to the sanitizer as crystalline sodium bromide dihydrate ($NaBr \cdot 2H_2O$).

Hypochlorite solutions were standardized in the conventional manner by titration with $Na_2S_2O_3$. Addition of $NaBr \cdot 2H_2O$ had no effect on the titration results.

Germicidal effectiveness was tested by the method of Weber and Black *(5)* at 25° C. By this method, the criterion for satisfactory performance of a sanitizer is 99.999% destruction in 30 sec., using suspensions containing 100 million bacteria per milliliter. Difco plate count agar was used for plating. All determinations were made in duplicate, at least.

A variety of bacteria were used as test organisms. The species included *Pseudomonas fluorescens*,[2] *Pseudomonas fragi*,[2] *Pseudomonas* species D-3,[3] *Escherichia coli* Co9,[3] *Micrococcus* species MS-102,[3] *Microbacterium flavum* OJ8,[4] and *Microbacterium lacticum* H2.[4] Plates of *Micrococcus* and *Escherichia* species were incubated at 35° C. for 48 hr., those of *Microbacterium* species at 30° C. for 72 hr., and those of *Pseudomonas* species at 20–22° C. for from four to five days.

Distilled water was used for all solutions and suspensions, unless stated otherwise.

Effect of different concentrations of $NaBr \cdot 2H_2O$. The *Micrococcus* and the two *Microbacterium* species were selected for the experiments, to determine the effect of different concentrations of $NaBr \cdot 2H_2O$ on the germicidal effectiveness of the sanitizer. The relatively high degree of resistance of these bacteria made it possible to study the germicidal effectiveness of the sanitizer over a wider range of hypochlorite and/or $NaBr \cdot 2H_2O$ concentrations and exposure times than would have been possible with less resistant bacteria. Results for *M. lacticum* H2 are presented (Table 1).

The results reveal that the effectiveness of the alkaline hypochlorite sanitizer was improved at any given concentration of available chlorine by addition of $NaBr \cdot 2H_2O$, and that the increase in effectiveness was in direct relationship to the concentration of $NaBr \cdot 2H_2O$.

By considering the points of satisfactory performance for each concentration of chlorine (less than 1,000 colonies after 30 sec. of exposure), one may observe that, percentagewise, less $NaBr \cdot 2H_2O$ was required to reach these points, with

[2] Supplied by Iowa State College, Department of Dairy Industry.
[3] Supplied by National Research Laboratories, Inc.
[4] Supplied by North Carolina State College, Department of Animal Industry.

TABLE 1

Effect of different concentrations of $NaBr \cdot 2H_2O$ on the germicidal effectiveness of a hypochlorite sanitizer of high alkalinity using Microbacterium lacticum H2 as test organism

Concentration of sanitizer	Concentration of $NaBr \cdot 2H_2O$ in sanitizer		Colony counts[1] Exposure time *(in seconds)*				
			15	30	60	120	300
	(%)	*(mM/l)*					
0.55 g/l or 5 p.p.m. available chlorine	0	0.0	TNTC	TNTC	TNTC	TNTC	TNTC
	1.35	0.015	TNTC	TNTC	TNTC	TNTC	TNTC
	2.7	0.03	TNTC	TNTC	TNTC	TNTC	TNTC
	4.05	0.045	TNTC	TNTC	TNTC	TNTC	TNTC
	5.4	0.06	TNTC	TNTC	5300	0	0
	6.75	0.075	TNTC	9900	30	0	0
	8.1	0.09	8600	100	0	0	0
	9.45	0.105	6500	300	0	0	0
0.31 g/l or 10 p.p.m. available chlorine	0	0.0	TNTC	TNTC	TNTC	TNTC	290
	1.35	0.03	TNTC	TNTC	TNTC	TNTC	40
	2.7	0.06	TNTC	TNTC	30	0	0
	3.38	0.075	TNTC	29000	60	....	....
	4.05	0.09	TNTC	890	0	0	0
	4.72	0.105	7400	10	0	0	0
	5.4	0.12	1500	0	0	0	0
	6.08	0.135	10	0	0	0	0
0.62 g/l or 20 p.p.m. available chlorine	0	0.0	TNTC	TNTC	TNTC	TNTC	3900
	0.27	0.012	TNTC	TNTC	TNTC	TNTC	50
	0.68	0.031	TNTC	TNTC	TNTC	100	0
	1.35	0.062	TNTC	TNTC	470	0	0
	2.03	0.093	TNTC	1700	100	0	0
	2.7	0.124	18000	100	130	0	0
	3.38	0.155	3400	10	0	0	0
	4.05	0.186	2300	0	0	0	0
1.24 g/l or 40 p.p.m. available chlorine	0	0	TNTC	TNTC	TNTC	TNTC	0
	0.39	0.031	TNTC	TNTC	TNTC	660	10
	0.78	0.062	TNTC	3600	0	0	0
	1.16	0.093	TNTC	3100	0	0	0
	1.35	0.124	TNTC	800	30	0	0
	1.74	0.155	TNTC	1700	0	0	0
	2.13	0.186	1300	30	0	0	0
	2.51	0.217	270	40	0	0	0
1.86 g/l or 60 p.p.m. available chlorine	0	0	TNTC	TNTC	TNTC	TNTC	0
	0.27	0.037	TNTC	TNTC	TNTC	0	0
	0.54	0.074	TNTC	TNTC	80	0	0
	0.81	0.11	TNTC	3000	0	0	0
	1.08	0.148	TNTC	2600	0	0	0
	1.35	0.186	TNTC	500	0	0	0
	1.62	0.223	12000	0	0	0	0
	2.16	0.260	1300	0	0	0	0
2.48 g/l or 80 p.p.m. available chlorine	0	0	TNTC	TNTC	TNTC	TNTC	0
	0.27	0.05	TNTC	TNTC	2600	0	0
	0.41	0.074	TNTC	TNTC	0	0	0
	0.54	0.099	TNTC	1500	0	0	0
	0.68	0.124	TNTC	2800	0	0	0
	0.81	0.149	TNTC	2800	0	0	0
	0.95	0.174	550	10	0	0	0
	1.08	0.198	500	0	0	0	0

[1] Counts below 1,000 after 30 sec. considered satisfactory.

increasing concentrations of chlorine. For example, approximately 8% of $NaBr \cdot 2H_2O$ was required at 5 p.p.m. of available chlorine to get satisfactory performance, but at 60 p.p.m. only 1.35% of $NaBr \cdot 2H_2O$ was required.

Another relationship is observed if the effectiveness of the mixtures is evaluated on the basis of the actual concentrations of $NaBr \cdot 2H_2O$ present in the solution. In this case, a larger amount of $NaBr \cdot 2H_2O$ is needed for higher concentrations of chlorine if satisfactory results are to be obtained. For example, at 5 p.p.m. of available chlorine, 0.09 m*M* of $NaBr \cdot 2H_2O$ per liter of sanitizer were required, but approximately twice that concentration (0.186 m*M* per liter) were required at 60 p.p.m. of available chlorine.

Similar trends were obtained, using *M. flavum* OJ8 and *Micrococcus* species MS-102. Only the degree of resistance of the three species differed.

Germicidal effectiveness of a standard mixture of sanitizer and $NaBr \cdot 2H_2O$. Under commercial working conditions, it would be impractical to combine the sanitizer and $NaBr \cdot 2H_2O$ in the proportions which would be considered most suitable for each existing condition. For this reason, a standard mixture was arbitrarily selected (98.65% sanitizer and 1.35% $NaBr \cdot 2H_2O$) and its germicidal effectiveness was compared to that of the sanitizer alone. The results are shown (Figure 1).

The bromide-hypochlorite mixture proved to be superior to the hypochlorite sanitizer itself, against all bacteria tested. The increases in effectiveness ranged from 33% against *M. flavum* OJ8 to 1,000% against *M. lacticum* H2. Increases in effectiveness against *E. coli* Co9 and the two species of *Pseudomonas* ranged from 200 to 400%. These results tend to confirm the observations that specific concentrations of $NaBr \cdot 2H_2O$ are more effective at low concentrations of available chlorine than at high concentrations. The variations in effectiveness of the bromide at different concentrations of available chlorine may be due to a pH effect on the effectiveness of the system.

A limited number of trials was conducted to compare the relative effectiveness of the alkaline sanitizer containing 1.35 and 4% of $NaBr \cdot 2H_2O$ to that of a commercial hypochlorite-type sanitizer of low alkalinity (pH 8).

The results demonstrated that the 1.35% mixture was about equal to the sanitizer of low alkalinity against the less resistant bacteria, *E. coli* and the species of *Pseudomonas*, but was of lesser effectiveness against the more resistant bacteria (Fig. 1). However, at a concentration of 4% $NaBr \cdot 2H_2O$, the effectiveness of the alkaline sanitizer was equal to that of the low alkaline sanitizer against the resistant bacteria.

Effect of organic matter, hard water, and temperature. The 1.35% mixture of $NaBr \cdot 2H_2O$ and the sanitizer was compared to the sanitizer by itself in the presence of 0.5% skimmilk and water of 200 p.p.m. hardness and at 5 and 45° C. The mixtures were superior to the sanitizer alone under all conditions, except in the presence of 200 p.p.m. hard water, when the effectiveness of the two sanitizers became equal. The variations in germicidal effectiveness, owing to the changed test conditions, were similar to those which have been found in other sanitizer studies *(1, 3)*.

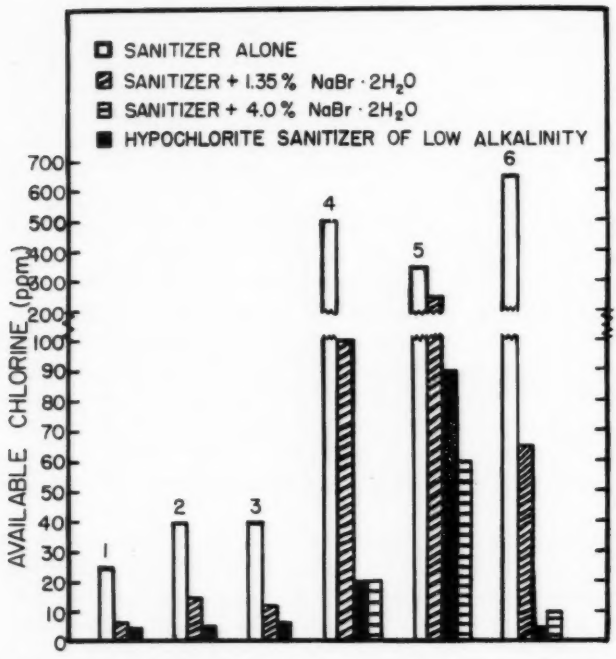

FIG. 1. P.p.m. available chlorine required for 99.999% destruction in 30 sec. of 1/*Pseudomonas* species D-3, 2/P. *fragi*, 3/*E. coli* Co9, 4/*Micrococcus* species MS-102, 5/*Microbacterium flavum* OJ8, and 6/*M. lacticum* H2. (High alkalinity sanitizer and mixtures containing NaBr · $2H_2O$, pH 11, low alkalinity sanitizer, pH 8 at 100 p.p.m. of available chlorine.)

Corrosion. Because of the possibility that the addition of NaBr · $2H_2O$ might have resulted in a change in the corrosive properties of the sanitizer, the corrosive actions of the sanitizer and the 1.35% mixture were compared. The hypochlorite sanitizer of low alkalinity also was included in this study.

Two methods were employed to determine corrosiveness of the sanitizers: a circulation and a quiescent procedure. In the former, solutions containing 200 p.p.m. of available chlorine were circulated over $\frac{5}{8}$ by 3 in. stainless steel strips, Types 302, 304, and 316, with a No. 2B finish. Circulation was continued for 7 hr. a day over a 2-wk. period. The strips were rinsed thoroughly with distilled water at the end of each day. In the quiescent method, $1\frac{1}{2}$ by 14 in. stainless steel bars, Type 304 with a No. 2B finish, were exposed to a solution of 200 p.p.m. of available chlorine over a period of 1 mo. The bars were submerged and exposed to air alternately for two days at a time. Corrosion was checked by weight in the circulation study and by visual inspection in the quiescent study.

None of the three sanitizers displayed measurable corrosiveness by the circulation method, and only the hypochlorite sanitizer of low alkalinity showed definite signs of being corrosive by the quiescent procedure. In the case of the

latter, heavy rust accumulations resulted at the air-liquid interface and slight pitting was evident on the bar.

CONCLUSIONS

The bactericidal effectiveness of a commercial hypochlorite-type sanitizer of high alkalinity is increased by the addition of $NaBr \cdot 2H_2O$. The increase in effectiveness at a given concentration of available chlorine is related to the concentration of $NaBr \cdot 2H_2O$ and/or to the test organism used.

The presence of 1.35% $NaBr \cdot 2H_2O$ does not affect the noncorrosive properties of the alkaline hypochlorite sanitizer at 200 p.p.m. of available chlorine.

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COMPARISON OF METHOXYL, LIGNIN, CRUDE FIBER, AND CRUDE PROTEIN CONTENTS OF FORAGE AND FECES AS INDIRECT INDICATORS OF DRY MATTER DIGESTIBILITY [1]

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SUMMARY

The correlation between methoxyl, lignin, and crude fiber contents of forage and feces and the digestible dry matter of the forage has been determined with 36 observations on forage samples and 65 observations on fecal samples (forage, −.818, +.300, and −.578, respectively; feces, −.851, −.087, and −.839, respectively). In subsequent work reported, the lignin determination was discontinued because it was not promising. The crude fiber determination was also discontinued, because it appeared to be no better as an indicator than the methoxyl determination, and the latter offered the technical advantages of measuring a precise chemical entity, and the procedure is less empirical.

With a total of 66 forage and 98 fecal observations, correlation coefficients of −.724 and −.725, respectively, were obtained between methoxyl content and the digestible dry matter of the forage. The protein content of the feces was a less reliable indicator of digestible dry matter, whereas that of forage had no indicator value (correlation coefficients of .528 and −.218, respectively).

The quantity of methoxyl present in forage materials appears to be closely related to the lignin content of the forage *(7, 13)* and both are inversely related to the dry matter digestibility. It is well known that as forage plants mature, their digestibility decreases and the percentages of lignin usually increases *(5–9, 15)*. Several workers *(4, 8, 9, 12, 16)* have reported the successful use of lignin as an indirect indicator of the digestibility of certain forages. However, Kane *et al. (11)* found this method less reliable with orchard grass than with alfalfa.

Lignin is not determined as a distinct chemical entity in the procedures usually employed in forage work but, rather, an "indigestible residue" is measured *(5, 6)*. All the proposed methods for lignin determination are laborious to perform and results are not easily reproduced. Methoxyl determinations do measure a distinct chemical entity and the procedure is relatively simple to perform.

With a limited number of observations, Richards and Reid *(15)* reported the correlation between methoxyl content of forage and digestible dry matter to be at least equal to that between lignin content in forage and digestible dry matter (−.991 and −.989, respectively). The methoxyl in the feces had a much higher correlation with digestible dry matter than did lignin in feces (−.974 and .425, respectively).

Because the methoxyl groups in forages are partly digested, Kane *et al. (10)* considered methoxyl to be unsuited for digestibility studies.

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Anthony *(1)* reported a high degree of correlation between methoxyl content and digestible dry matter (−.742). However, the scatter of observations around the regression line was rather great.

The study reported herein was conducted to compare further the reliability of methoxyl, lignin, crude fiber, and crude protein contents in forage and fecal materials as indirect indicators of forage digestibility.

EXPERIMENTAL PROCEDURE

The data were obtained from several different digestion studies in which the forage under study was the sole ration. Some of the forages were hand-fed and from these the relationships of forage constituents to digestibility were determined. Other trials included freely grazing animals in which the chromogens method *(14)* was used to determine the forage digestibility. With these, corresponding forage samples could not be obtained. In most cases, simultaneous conventional digestion trials were run, the animals being fed forage clipped from the same field. Good agreement between the chromogens and conventional methods was usually obtained. The fecal relationships were determined, using samples from both conventional trials and the trials in which digestibility was determined by the chromogens method.

The lignin determinations were made by the method of Ellis *et al. (6)*, as modified by Thacker *(18)*; however, the incubation with Na_2CO_3 was omitted. Methoxyl was determined by a modification of the Zeisel method, as outlined by the U. S. Forest Products Laboratory *(3)*. Moisture determinations were made by toluene distillation *(2)*. Crude fiber and nitrogen were determined by the methods suggested by the Association of Official Agricultural Chemists *(2)*.

For the forage relationships, the following forages were used: 11 alfalfa lots grown in four seasons with 42 conventional digestibility determinations; five Sudan grass lots grown in four seasons with 15 conventional digestibility determinations, and three orchard grass lots grown in one season with nine conventional digestibility determinations. In all, there were 19 forages fed to 66 animals.

The relationships of fecal constituents to digestible dry matter were obtained from the digestion trials indicated above and from the following grazing trials in which digestibility was determined using Reid's chromogen technique *(14)*: 14 alfalfa determinations from five different lots of alfalfa; nine Sudan grass determinations from three lots of Sudan grass, and nine orchard grass determinations from three lots of orchard grass. Total number of samples was 98.

RESULTS AND DISCUSSION

The average compositions of the various alfalfa, Sudan grass, and orchard grass forages fed, and of the resulting feces, are shown (Table 1).

Following the 1954 digestion trials, correlation coefficients between digestible dry matter and each of the following constituents in the forage and in the feces were calculated *(16)*: methoxyl, lignin, and crude fiber. The correlation coefficients are shown (Table 2).

TABLE 1

Composition of forage and feces

Growth stage	No. of observations	Methoxyl	Lignin	Crude protein	Crude fiber	Digestible dry matter
				(%)		
1952			Alfalfa			
Very early bloom						
Forage	3	2.70	13.2	21.9	30.6	56.5
Feces	5	3.06	28.4	15.2	39.3	58.6[a]
One-half bloom						
Forage	3	2.37	13.6	19.7	32.3	56.8
Feces	6	3.10	27.5	15.4	39.1	60.0
Full bloom						
Forage	3	2.54	14.1	19.4	35.4	54.3
Feces	6	3.52	29.2	13.5	43.6	55.5
1953						
Early bud						
Forage	3	1.79	14.9	19.8	28.6	59.6
Feces	6	2.51	25.5	12.3	34.2	60.2
Very early bloom						
Forage	3	2.06	12.8	20.2	30.7	60.3
Feces	6	2.98	27.7	12.8	39.4	61.7
1955						
Very early bloom						
Forage	4	2.16	.....	15.1	.....	62.5
Feces	4	2.81	.....	11.8	.....	62.5
One-half bloom						
Forage	4	2.30	.....	13.8	.....	57.2
Feces	4	2.90	.....	11.2	.....	57.2
Full bloom						
Forage	4	2.38	.....	14.6	.....	59.6
Feces	4	3.00	.....	11.6	.....	59.6
1956						
Very early bloom						
Forage	5	2.28	.....	16.2	.....	63.6
Feces	5	3.11	.....	12.1	.....	63.6
One-half bloom						
Forage	5	2.42	.....	14.5	.....	57.0
Feces	5	3.27	.....	10.8	.....	57.0
Full bloom						
Forage	5	2.42	.....	17.7	.....	60.3
Feces	5	3.35	.....	11.2	.....	60.3
1952			Sudan grass			
Very few flower stalks						
Forage	3	1.75	11.0	7.5	32.2	69.5
Feces	3	2.83	28.7	13.0	25.1	69.5
About 25% flower stalks						
Forage	3	1.77	9.7	10.8	32.5	65.4
Feces	3	2.84	25.6	10.4	29.7	65.4
1953						
Some in head						
Forage	3	1.62	13.8	13.9	29.3	68.0
Feces	6	2.12	25.3	14.0	19.8	70.3
1954						
Some in head						
Forage	3	1.78	13.0	18.5	29.4	67.4
Feces	6	2.49	27.1	14.4	22.8	69.5
1955						
Some in head						
Forage	3	2.05	.....	10.2	.....	63.6
Feces	6	2.88	.....	14.7	.....	65.7
1954			Orchard grass			
Prebloom						
Forage	3	1.63	20.8	14.7	32.5	64.0
Feces	6	2.56	27.3	14.6	28.1	64.8
Full bloom						
Forage	3	2.03	12.8	11.8	36.5	56.6
Feces	6	3.05	29.0	11.2	33.9	58.2
Early seed						
Forage	3	2.41	10.4	7.5	38.2	53.1
Feces	6	3.48	23.4	8.9	36.3	53.3

[a] The number of observations differs from those for forage because grazing trials in which digestible dry matter was determined by Reid's chromogens method are included.

The correlations between lignin and digestible dry matter were significant only with the orchard grass forage, and this was a positive correlation rather than the negative one which was expected. The correlation of lignin with digestible dry matter in feces was positive in the orchard grass trials and negative in the alfalfa trials. These results cast considerable doubt on the predictive value of lignin.

TABLE 2

Correlation coefficients of methoxyl, lignin, and crude fiber with digestible dry matter (1952—1954)

Feed	No. of observations	Methoxyl	Lignin	Crude fiber
		Forage		
Alfalfa	15	−.736[a]	−.0744	−.745[a]
Orchard grass	9	−.973[a]	+.985[a]	−.990[a]
Sudan grass	12	−.375	+.280	−.117
Combined	36	−.818[a]	+.300	−.578[a]
		Feces		
Alfalfa	29	−.921[a]	−.616[a]	−.749[a]
Orchard grass	18	−.837[a]	+.481[b]	−.745[a]
Sundan grass	18	−.595[a]	+.159	−.767[a]
Combined	65	−.851[a]	−.087	−.839[a]

[a] Highly significant.
[b] Significant.

The crude fiber and methoxyl contents of both forage and feces were highly correlated with digestible dry matter in all cases except in the Sudan grass forage. The Sudan grass trials did not cover a wide range of plant maturity, whereas both the orchard grass and alfalfa trials did cover such a range.

Because the prediction of dry matter digestibility from the lignin content of forage or feces appeared much less promising than that from either crude fiber or methoxyl contents, lignin determinations have not been made on samples collected since 1954. Inasmuch as methoxyl in feces was at least equal to that of crude fiber in feces, and methoxyl in forage was considerably superior to crude fiber in forage as an indicator of dry matter digestibility, and because the determination of methoxyl is more precise than that for crude fiber, emphasis has subsequently been upon methoxyl content.

The correlation coefficients of methoxyl and crude protein content in forage and feces with digestible dry matter for all trials, 1952–1956, are shown (Table 3). The crude protein of the forage had no predictive value, whereas that of feces was quite good for orchard grass and Sudan grass but of no value for alfalfa. The methoxyl content continued to show a highly significant negative correlation with digestible dry matter in both forage and feces.

Regression of digestible dry matter on forage methoxyl and fecal methoxyl, respectively, are shown (Figures 1 and 2). There is considerable scatter of data in both figures. The alfalfa trials furnished the largest portion of the data used in the calculation of these regression lines; therefore, it is not surprising that the regression line calculated from feces data appears to fit the alfalfa data better than it does either the Sudan grass or the orchard grass data. There are two few data at both the high and low ends of the regression lines to have a very great

TABLE 3

Correlation coefficients of methoxyl and crude protein with digestible dry matter (1952—1956)

Forage	Forage			Feces		
	No.	Methoxyl	Crude protein	No.	Methoxyl	Crude protein
Alfalfa	42	−.426[a]	−.224	56	−.498[a]	.222
Orchard grass	9	−.973[a]	.499	18	−.837[a]	.867[a]
Sudan grass	15	−.661[a]	.022	24	−.743[a]	.570[a]
Combined	66	−.724[a]	−.218	98	−.725[a]	.528[a]

[a] Highly significant.

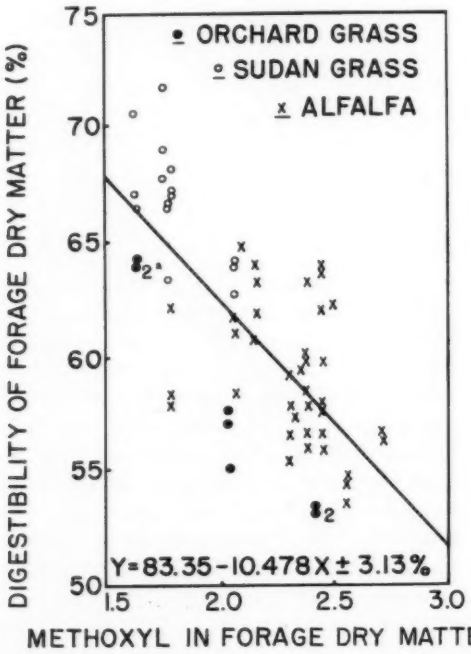

FIG. 1. Relation of methoxyl level in forage to forage digestibility.

[a] 2. This point represents two observations.

effect upon the slope of the feces regression line; however, those available are above the line at the high end and below it at the low end. If this trend were to continue with additional data, a regression line covering the entire range would be steeper than the present line. Inasmuch as the Sudan grass data are mainly above the line at the high end, and the orchard grass data are mainly below the line at the low end, a regression line with greater slope would tend to fit these data better. Some of the same trend can be noted in the regression line for forage methoxyl, but it is much less apparent.

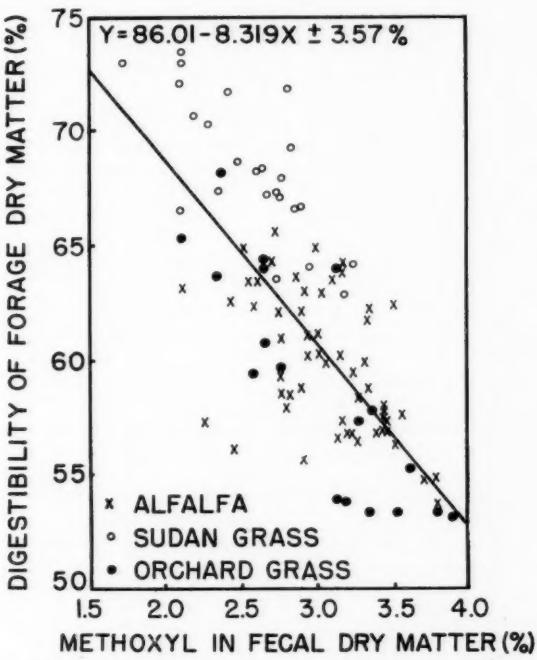

FIG. 2. Relation of methoxyl level in feces to forage digestibility.

The regression equations and their standard errors of estimate are also given in the figures. These data indicate that, with the forages studied, there is a high degree of correlation between the methoxyl content of forage and feces and the digestible dry matter of the forages.

Although the scatter of data appears to be too great to allow use of methoxyl content, when precise digestion values are required, the determination of methoxyl content in forage and/or feces would provide a relatively simple method for ranking and/or screening the quality of forages.

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COMPOSITION OF CRUDE FIBER IN CERTAIN ROUGHAGES[1]

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SUMMARY

The lignin and cellulose contents of the dry matter of 11 roughages have been determined. These included two legume hays, five grass hays, oat straw, and oat silage made from plants harvested at the boot, milk, and dough stages of growth. The cellulose content of the grass hays and Korean lespedeza hay was higher than that of alfalfa hay. The lignin content of the roughages was quite variable, probably due to differences in stage of growth of the plants when harvested. The cellulose content of the crude fiber was much higher in the grass hays than in the legume hays. The lignin content in the crude fiber of the legume hays was greater than in the grass hays. The findings of earlier workers, that the composition of crude fiber varied as between species, have been confirmed. It has been shown that in the oat plant the crude fiber make-up is dependent on the stage of growth.

The most generally accepted method of analyzing for carbohydrates in roughages is to determine the so-called crude fiber and to use a difference fraction, the nitrogen-free extract *(2)*. Crude fiber is supposed to represent the less readily available portion of the feed and nitrogen-free extract to represent the more easily digestible carbohydrates. Numerous workers *(1, 11, 13, 17, 19, 20, 34)* have shown that crude fiber is of variable composition, consisting mainly of celluloses, lignin, hemicelluloses, pentosans, and small amounts of crude protein and ash. These constituents appear in different ratios in various species and are also influenced by the stage of maturity of the plant *(1)*.

Nordfeldt *et al. (18)* found that the nitrogen-free extract fraction was composed of cellulose, lignin, pentosans, hexosans, sugars, organic acids, and other compounds in lesser concentrations. Crampton and Maynard *(5)*, using Morrison's data *(16)*, found that in dry roughages 39% of the coefficients of apparent digestibility listed showed as great or greater coefficients of digestibility of crude fiber as the nitrogen-free extract. Moreover, investigations of the digestibility of grasses and their products have shown that in many materials the crude fiber has a higher digestibility than the nitrogen-free extract *(34, 35)*. Bondi and Meyer *(3)* observed that large percentages of the pentosans and lignin were found in the nitrogen-free extract in both plants and feces; while most of the hexosans were found in the crude-fiber fraction of both plants and feces. Nordfeldt *et al. (18)* observed that 40.6% of the total lignin in a hay was contained in the crude fiber fraction, and that the remaining 59.4% appeared in the nitrogen-free extract. In oat straw, however, much more of the lignin was present in the nitrogen-free extract than in the crude fiber. Bondi and Meyer *(4)* made a comprehensive study of lignins from various plant sources. Both the nitrogen and methoxyl content of lignin varied, depending on the plant

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source. Ely *et al. (7)* found that the nitrogen associated with lignin in orchard grass decreased as the plant became more mature. Numerous workers *(7, 10, 19, 22, 23, 25, 27–30, 33)* have found that the stage of maturity influences the composition of plants. The cellulose, crude fiber, lignin, methoxyl, and hemicellulose contents tend to increase with maturity. The digestibility of crude fiber, nitrogen-free extract, and cellulose tends to decrease with increasing maturity.

The objective of this study was to determine the composition of the crude fiber of some hays and silages utilized in feeding dairy cattle.

EXPERIMENTAL PROCEDURE

Several roughages commonly used in the southwestern United States were selected for the study. One lot of U. S. No. 1 Grade alfalfa hay was used that was made from plants harvested when in early-bloom stage. The lot of Korean lespedeza hay was made from plants harvested in early-bloom stage. The millet hay was made from plants of the cat-tail variety, harvested in the boot stage. The Bermuda grass hay was made from pasture clippings of common Bermuda grass. The plants were growing on soil that had received heavy amounts of both urine and manure and were of very high quality. The fescue hay was made from plants of the Kentucky 31 Variety, harvested in the full-head stage. The prairie hay used was purchased in eastern Oklahoma. It was of mixed species and of high quality. The oat straw used was obtained from plants of the Arkwin Variety, a winter oat grown both for pasture and for grain production. Silage was made from oat plants of the Arkwin Variety that were ensiled in the boot, milk, and dough stages of growth. These silages have been described in detail elsewhere *(33)*.

All roughages were analyzed for crude protein, ether extract, crude fiber, and ash, as outlined by the A.O.A.C. methods *(2)*. Nitrogen-free extract was determined by calculation of the difference between the total of the previously stated analyses and 100%. Cellulose was determined by the method of Crampton and Maynard *(5)*, and lignin by the method of Ellis *et al. (6)*.

Large samples of crude fiber were prepared from all the roughages studied. The technique used was that of the A.O.A.C. methods *(2)* except that, instead of being ashed for determination, the fiber was dried in an oven at 60° C., then analyzed for cellulose, lignin, crude protein, and ash.

RESULTS AND DISCUSSION

Data pertaining to the analyses of the dry matter of the samples studied are presented (Table 1). The crude fiber was, as a rule, higher in the grasses than in the legumes. The protein content was high in the leguminous hays and in orchard grass and Bermuda grass hays. The data concerning the oat silages demonstrate the influence of advancing maturity on protein content. The high yield of grain tends to effect a trend somewhat different from that of other plants, as indicated by a decrease in crude fiber content and cellulose content from the milk to dough stage of maturity, and an increase in nitrogen-free extract.

TABLE 1
Analysis of the dry matter of roughages studied

Roughage	Percentage of dry matter						
	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract	Ash	Lignin	Cellulose
Korean lespedeza hay	11.45	2.24	33.64	48.81	3.86	13.20	32.62
Orchard grass hay	16.09	3.37	31.71	40.48	8.35	6.96	29.86
Bermuda grass hay	16.55	2.63	30.47	39.72	10.63	10.05	33.25
Kentucky 31 fescue hay	5.95	1.10	44.33	43.64	4.98	11.38	40.50
Alfalfa hay	17.03	2.36	31.76	41.85	7.00	7.88	26.22
Millet hay	8.75	1.91	38.18	42.40	8.76	8.88	38.30
Oat straw	4.12	2.20	50.87	37.59	5.22	12.97	41.65
Prairie hay	5.88	2.24	36.32	49.41	6.15	8.79	32.97
Oat silage (boot)	13.66	2.61	30.18	46.91	6.64	8.61	33.71
Oat silage (milk)	12.36	3.42	32.24	45.51	6.47	15.30	33.06
Oat silage (dough)	11.16	2.05	28.92	52.15	5.72	8.56	28.99

The grasses had a higher cellulose content than did alfalfa hay. The lignin content was higher in lespedeza hay, oat straw, alfalfa hay, and prairie hay than in the other dry roughages. The lignin content of the oat silage made from plants in the milk stage was higher than of the silages made from oat plants in the boot stage of maturity. The lignin content of the oat silage decreased from the milk to dough stage. Apparently this is due to grain formation *(15)*. The ratio of lignin to cellulose is much narrower in the legumes than in the grasses. The ratio of lignin to crude fiber is also closer.

Results of analysis of the samples of crude fiber isolated from the roughages utilized in this study are presented (Table 2). Various methods have been used to present the composition of crude fiber. Armstrong *et al. (1)* have shown that crude fiber consists almost wholly of cellulose, lignin, and crude protein when expressed on an ash-free dry matter basis. Our data, however, conform more nearly to those of Nordfeldt *et al. (18)*, who found that the crude fiber of a hay contained, on the percentage basis: cellulose, 80.1; lignin, 11.5; pentosans, 10.9, and small amounts of crude protein and ash. The cellulose, lignin, crude protein, and ash accounted for from 89 to 101% of the total of crude fiber, depending on the roughage. The difference may be due to pentosans as found by Nordfeldt

TABLE 2
Analysis of crude fiber

Roughage	Percentage of dry matter				
	Cellulose	Crude lignin	Crude protein	Ash	Total
Korean lespedeza hay	70.24	19.87	1.90	1.18	93.19
Orchard grass hay	84.63	8.80	1.28	1.90	96.91
Bermuda grass hay	70.77	10.70	1.62	2.37	94.46
Kentucky 31 fescue hay	80.98	7.96	0.63	2.06	91.63
Millet hay	79.98	6.89	0.72	1.62	89.21
Alfalfa hay	67.41	17.61	2.58	2.31	97.48
Prairie hay	85.95	8.61	0.39	0.04	94.99
Oat straw	80.01	10.63	0.65	0.09	91.38
Oat silage (boot)	89.33	8.96	1.03	1.75	101.07
Oat silage (milk)	72.20	8.71	2.42	1.47	84.80
Oat silage (dough)	79.51	9.32	2.36	4.13	95.32

et al. (18), or to some other carbohydrate fraction not determined in this study. In the oat silage made from plants in the boot stage, cellulose, lignin, crude fiber, and ash accounted for all the crude fiber, but silage made from later stages of growth did not—a fact noted by others *(1)*. The cellulose content of the crude fiber was higher in the grasses than in the legumes. The per cent of lignin in the crude fiber was about twice as great in the legumes as in the grasses. This fact has also been observed previously *(1, 19)*. The percentages of crude protein and ash present in the isolated crude fiber was low in all cases. They are presumably trapped by the encrustation of plant tissues by lignin *(1, 19)*.

The percentages of cellulose, lignin, and crude protein recovered in crude fiber, expressed as a percentage of that in the original material are presented (Table 3). The percentage recovery of cellulose lay within a comparatively small range (70.41–97.72%); the lignin recovery was much more variable (18.35–70.98%). These data indicate that in some plants apparently more of the lignin appears in the nitrogen-free extract fraction than in the crude fiber fraction. The percentage of the total crude protein of the plant material that was recovered in the crude fiber was of a low magnitude.

Norman *(21)* and Bondi and Meyer *(4)* also have pointed out that there is little justification for the view that lignin is uniform and homogenous, and that the lignin of different species is not identical. Ely *et al. (7)* have shown that the nitrogen and methoxyl contents of the lignin of orchard grass change as maturity advances.

It has been shown that crude fiber does not represent a single plant constituent, and that its composition varies to a considerable extent as between species and was seen to vary in the oat plant as the stage of maturity advanced. It follows from the foregoing, and from the analysis of lignin isolated from these forages, that nitrogen-free extract also varies in composition. From a scientific point of view, especially in feeding to determine the causes of small differences in digestibility, such a state of affairs can not be regarded as entirely satisfactory. It is true that crude fiber is a good criterion of the digestibility of organic matter *(8, 12, 14, 26)*. Nevertheless, there is evidence that lignin may be an even better

TABLE 3

Percentage of cellulose, lignin, and crude protein recovered in crude fiber

Roughage	Percentage of that in original material		
	Cellulose	Lignin	Crude protein
Korean lespedeza hay	72.44	50.64	5.60
Orchard grass hay	89.87	40.09	2.52
Bermuda grass hay	73.10	32.42	2.98
Kentucky 31 fescue hay	88.62	31.01	4.69
Alfalfa hay	81.52	70.98	4.81
Prairie hay	94.68	35.58	2.41
Oat straw	97.72	41.69	8.02
Millet hay	79.73	29.62	3.14
Oat silage (boot stage)	79.98	31.41	2.28
Oat silage (milk stage)	70.41	18.35	6.47
Oat silage (dough stage)	79.32	31.49	6.11

criterion *(5, 9, 31, 32)*. This, together with a separation of the carbohydrate fraction into its chemical entities, would seem to merit consideration.

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RELATION OF LACTATION MILK PRODUCTION IN DAIRY COWS TO MAXIMUM INITIAL MILK YIELD AND PERSISTENCY OF LACTATION[1, 2]

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SUMMARY

A correlation study was performed on "total," "among-lactations," and "among-cows-in-lactation" bases, with 139 lactation records from 93 Holstein cows and 57 lactation records from 34 Guernsey cows, to determine the effects of maximum initial milk yield and persistency of lactation on lactation milk production. Since the relationships among these factors were found to be similar in the two breeds, combined correlations were obtained on an "among-lactations-in-breed" and "among-cows-in-lactation-in-breed" basis. Maximum initial milk yield and persistency were highly correlated with lactation milk production (0.81[**][3] and 0.32[**], respectively) on an "among-cows-in-lactation-in-breed" basis; whereas, they had no correlation with each other (0.04), suggesting that maximum initial milk yield and persistency were independent factors which collectively influence lactation milk production in a positive way ($R = 0.86^{**}$). On an "among-lactations-in-breed" basis, lactation milk production and persistency were negatively correlated (-0.72^{**}), which expresses the observed effects of age of maturity on this relationship.

In an analysis of the variation association with lactation milk production on an "among-cows-in-lactation-in-breed" basis, the effects associated with the correlation of maximum initial milk yield accounted for 65.6% of the total variation; whereas, the additional effects due to persistency contributed 8.5% to the total variation. Similarly, on an "among-lactations-in-breed" basis, the effects associated with the correlation of maximum initial milk yield with lactation milk production accounted for 92.1% of the total variation, and the effects due to persistency contributed an additional 0.9% to the total variation.

Many studies *(2, 4, 7, 9, 10, 13, 14, 17)* have attempted to determine the relationships which exist among lactation milk production and its components, maximum initial milk yield and persistency of lactation. This is an important area of study, especially as it relates to physiological relationships. However, a difficult analytical problem has presented itself in these studies. As a cow advances in age, body weight, or lactation number, both lactation milk production and maximum initial milk yield increases *(18)*; whereas, the persistency of lactation by most common measures decreases *(7, 12, 18)*. Thus, from a total of over-all viewpoint, the age, body weight, or lactation number would be positively correlated with lactation milk production and maximum initial milk yield, and at the same time negatively correlated with the persistency of lactation.

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[2] This study was part of a Northeast Regional Project (NE-30, The Relation of Certain Physiological Factors to the Productive Capacity of Dairy Cattle), a cooperative study involving agricultural experiment stations in the Northeastern Region and supported in part by regional funds of the United States Department of Agriculture.

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Much more important from a physiological viewpoint are the relationships which exist among the lactation milk production components in animals of similar age or under conditions in which the effects of aging, body weight, or advance in lactation number are eliminated or adjusted. In this regard, partial correlation techniques *(4, 7, 13)* have been used in which the effects of age or body weight have been held constant. In other studies *(4, 10, 14)*, the lactation production data have been corrected for age before applying correlation techniques.

A different approach to the age-correction problem may be made, in which lactation records are grouped into classes by lactation number. The coefficients of correlation then may be derived on "total," "among-lactations," and "among-cows-in-lactation" bases. The "among-lactations" correlations will represent the effects of aging or increase in body weight (as represented by lactation number) on the various relationships. The "among-cows-in-lactation" correlations will represent the effects among cows, independent of age or body weight, and the use of correlations on this basis eliminates the need for prior correction of lactation records for the effect of age. In addition, the "among-cows-in-lactation" basis lends itself to correlating lactational characteristics with other characteristics of physiological significance (*e.g.*, thyroid activity), which also may need to be age-corrected. It is clear that the correlations based on "total" are merely an average of the "among-lactations" and "among-cows-in-lactation" effects and would be of relatively little significance or importance.

If more than one breed of dairy cattle is being studied, and the relationships among the components of lactation milk production are found to be similar, then the correlations for the breeds may be combined on an "inbreed" basis.

This study embodies the above principles in determining the relationships which exist among lactation milk production and its components, maximum initial milk yield and persistency of lactation. If these components are independent factors which individually and collectively are important in influencing lactation milk production, they will be part of subsequent physiological studies involving lactation milk production.

EXPERIMENTAL PROCEDURE

For this study, 139 lactation records from 93 Holstein cows and 57 lactation records from 34 Guernsey cows on twice-a-day-milking were collected during the period from 1955 to 1958, from the experimental herd of the Dairy Research Farm, New Jersey Agricultural Experiment Station, Sussex.

Calculations of monthly and lactation milk production were based solely on test-day data collected once a month. Equal quantities of morning and evening milk on test-day were combined and a single Babcock fat test was made. Monthly milk yields were calculated on a 4% FCM basis *(10)* and accumulated to 240 days. The 240-day 4% FCM production constituted lactation milk production, expressed on an energy basis and largely independent of the effects of pregnancy. Maximum initial milk yield was calculated as the highest test-day 4% FCM production. Estimates of persistency were based on the findings of

Brody *et al. (1)* and Gaines *(2)*, that an exponential equation, $M_t = M_o e^{-kt}$, best describes the decline in milk production with the advance in lactation, in which M_t equals milk production during any month, t; M_o equals the theoretical level of milk production at the time of parturition; e equals the base of natural logarithms, and k is a constant representing the rate of decline in milk production per month as proportional to the rate of monthly yield at the moment (*i.e.*, on an instantaneous basis.)

The use of k as a measure of persistency has been criticized *(7)* because it is negatively correlated with actual persistency; hence is confusing in its interpretation when used in correlation studies. This objection is readily overcome by the use of the following transformation: persistency index $(P) = 100 - (k \times 100)$ in which P is now positively correlated with actual persistency and is expressed on a percentage basis. The persistency index as described was used in this study.

A detailed illustration of the machine method of calculating the persistency index *(P)* is as follows:

1. The method of least squares is used to calculate the persistency index, based on the milk production of the last six test days of a 240-day lactation period in which each test-day milk yield was converted to a 4% FCM basis.

2. An example of the tabular layout *(8)* follows, in which X-values are coefficients representing the six test-days and coded to avoid correction factor calculations, Y-values are the pounds of 4% FCM yield on each of the last six test-days of a 240-day lactation, and Y′-values are the common logarithms of the pounds of 4% FCM. The example is based on first-lactation data of Holstein 165.

X	Y	Y′
5	30.2	1.4800
3	29.8	1.4742
1	29.5	1.4698
−1	20.9	1.3202
−3	15.9	1.2014
−5	12.0	1.0792

3. The sum of products of X and Y′ (equals $\Sigma xy'$) are accumulated on a calculator, using negative multiplication for the three negative XY′-products. In the example, $\Sigma xy' = 2.9720$.

4. The regression coefficient, k, is calculated from the following formula:

$$k = \frac{(\Sigma xy')\,(2)\,(2.3)}{70} = (\Sigma xy')\,(0.0657)$$

The 2 is due to the interval of X being two units, the 2.3 is a factor converting common to natural logarithms, and 70 is $\Sigma X^2 = \Sigma x^2$, since the mean of $X = 0$. The factor, 0.0657, combines these terms and is a constant for all calculations where six test-day 4% FCM values are used.

5. From the example, $k = 2.9720 \times 0.0657 = 0.195$.

6. Persistency index $(P) = 100 - (k \times 100)$, or from the example,

$$P = 100 - (0.195 \times 100) = 80.5\%.$$

Lactation records were segregated according to lactation number. Body weights were determined on all animals at 30 days postpartum and were considered to be the lactation body weight. The data from each breed were subjected to a correlation analysis on "total," "among-lactations," and "among-cows-in-lactation" bases, using the method of least squares *(15)*.

RESULTS AND DISCUSSION

Table 1 presents the mean lactation record characteristics, grouped according to breed and lactation number. Body weight, 240-day 4% FCM production, and maximum initial 4% FCM yield all tend to increase with advance in lactation number, the reverse being true for the persistency index.

Table 2 presents the coefficients of correlation among lactational characteristics for each breed on an "among-lactation" and "among-cows-in-lactation" basis. Since the relationships among the characteristics were similar in both breeds, the original data were combined and the coefficients of correlation also presented on an "in-breed" basis (Table 2).

The "among-lactations-in-breed" coefficients of correlation indicate the nature of relationships among the various factors due to increasing age or body weight. They are the same as those which would be obtained by correlating the means of the various characteristics derived on a lactation number or age basis. A high positive correlation will be noted (Table 2) between 240-day 4% FCM production and maximum initial 4% FCM yield (0.96**).[3] This relationship was first indicated by Gavin *(5)* and later substantiated by others *(4, 7, 13)*. Two

TABLE 1

Lactation record characteristics classified by lactation number based on twice-a-day milking

Lacation No.	Breed	Number of lactation records	Lactation record characteristics			
			Body weight	240-day 4% FCM production	Max. initial 4% FCM	Persistency index
			(lb.)			*(%)*
1	Holstein	36	1,138	8,043	39.0	95.0
	Guernsey	13	947	6,761	34.4	95.1
2	Holstein	30	1,234	9,568	52.9	88.5
	Guernsey	10	1,086	7,734	46.6	87.4
3	Holstein	17	1,296	11,733	63.1	89.4
	Guernsey	6	1,109	9,348	50.7	88.5
4	Holstein	19	1,311	10,811	59.1	87.1
	Guernsey	8	1,143	8,311	45.9	83.0
5	Holstein	16	1,360	11,089	64.5	87.5
	Guernsey	9	1,122	9,131	51.0	89.5
6	Holstein	12	1,453	11,327	66.8	83.9
	Guernsey	7	1,181	9,239	47.9	88.7
7	Holstein	3	1,377	10,792	65.3	77.8
	Guernsey	3	1,176	9,234	50.1	89.8
8	Holstein	2	1,376	11,201	60.7	83.9
	Guernsey	1	1,048	7,948	55.1	86.2
9	Holstein	2	1,397	12,225	64.4	80.8
10	Holstein	2	1,187	10,343	56.5	80.9
Total or mean	Holstein	139	1,267	10,034	54.5	89.1
	Guernsey	57	1,102	8,463	47.7	88.5

TABLE 2

Coefficients of correlation among lactational characteristics in Holstein and Guernsey cows on separate breed and "in-breed" bases

Characteristics correlated	Coefficients of correlation and degrees of freedom					
	Among lactations			Among cows in lactation		
	Holsteins	Guernseys	In breed (combined)	Holsteins	Guernseys	In breed (combined)
Zero-order correlations—degrees of freedom	8	6	15	129	49	179
240-day 4% FCM *versus* maximum initial 4% FCM	0.97**	0.89**	0.96**	0.81**	0.79**	0.81**
240-day 4% FCM *versus* persistency index	−0.77**	−0.52	−0.72**	0.29**	0.42**	0.32**
Maximum initial 4% FCM *versus* persistency index	−0.87**	−0.68	−0.81**	−0.02	0.19	0.04
Partial correlations—degrees of freedom	7	5	14	128	48	178
240-day 4% FCM *versus* max. initial 4% FCM independent of persistency index	0.97**	0.86*	0.92**	0.87**	0.42**	0.84**
240-day 4% FCM *versus* persistency index independent of max. initial 4% FCM	0.59	0.27	0.34	0.52**	0.44**	0.50**
Multiple correlations—degrees of freedom	7	5	14	128	48	178
240-day 4% FCM with max. initial 4% FCM and persistency index	0.98**	0.90**	0.96**	0.87**	0.84**	0.86**

* * and ** indicate statistical significance at the 5% ($P < 0.05$) and 1% ($P < 0.01$) levels, respectively.

hundred and forty–day 4 % FCM production was negatively correlated with persistency index (−0.72**), agreeing in this respect with other investigations *(4, 9)*. The multiple correlation of 240-day 4% FCM production with maximum initial 4% FCM yield and persistency index on an "among-lactations-in-breed" basis was 0.96.**

The "among-cows-in-lactation-in-breed" coefficients of correlation (Table 2) are especially important, and they present relationships which are essentially independent of effects due to age or maturity. On this basis, both maximum initial 4% FCM yield and persistency index were highly correlated in a positive manner with 240-day 4% FCM production (0.81** and 0.32,** respectively), indicating that in any given lactation, cows attaining a greater total lactation production record tend to start lactation at a higher level of production and to be more persistent. These findings substantiate those of Gaines *(4)* who found similar first-order partial correlations independent of the age of the animals, and of Mahadevan *(10)* and Sikka *(14)*, who reported similar relationships using age-corrected data on a "between-cows-within-herd" basis. The coefficient of correlation (among-cows-in-lactation-in-breed) between maximum initial 4% FCM yield and persistency index was not statistically significant (0.04), indicating that these two characteristics are independent factors, each exerting a separate, positive influence on 240-day 4% FCM production. Sikka *(14)* also noted a zero correlation between these two characteristics. This may indicate that maximum initial milk yield and persistency of milk production are inherited as separate factors, as suggested by Gilmore *et al. (6)*, and may be influenced in a differential manner by physiological factors such as thyroid activity.

The foregoing observations show that, although as animals mature and produce more milk both initially and totally in successive lactations, persistency nevertheless declines. In any given lactation, however, higher total milk production is associated with higher initial milk yield and also with higher persistency, the latter two factors being expressed independently of each other.

An assessment of the relative importance of maximum initial milk yield and persistency on lactation milk production is presented (Table 3). The squares of the coefficients of correlation (r^2 or $R^2 \times 100$) were used to determine the per cent of total variation in lactation milk production attributable to these two associated factors.

On an "among-cows-in-lactation-in-breed" basis, the effects due to correlation of maximum initial milk yield with lactation milk production (0.81) accounted for 65.6% ($0.81^2 \times 100$) of the total variation associated with lactation milk production. Similarly, the effects due to the multiple correlation of both maximum initial milk yield and persistency with lactation milk production (0.86) accounted for 74.0% ($0.86^2 \times 100$) of the total variation in lactation milk production. Thus, the additional effect of persistency on lactation milk production was 8.4% (74.0–65.6%) of the total variation in lactation milk production. These evaluations of effects are in general agreement with those reported by Gooch *(7)* and Sikka *(14)*. Thus, lactation milk production on an "among-cows-in-lactation-in-breed" basis was influenced primarily by maximum initial milk yield

TABLE 3

Effects of maximum initial milk yield and persistency of lactation on the variation associated with lactation milk production

Source of variation	Per cent of total variation	
	Among lactations	Among cows in lactation
	(%)	
Effects due to:		
Correlation of max. initial 4% FCM with 240-day 4% FCM		
Holsteins	94.8	66.3
Guernseys	79.5	62.7
In breed (combined)	92.1	65.6
Additional effect of correlation of persistency index with 240-day 4% FCM		
Holsteins	1.8	9.3
Guernseys	1.5	7.4
In breed (combined)	0.9	8.4
Random or error effects		
Holsteins	3.4	24.4
Guernseys	19.0	29.9
In breed (combined)	7.0	26.0

(65.6%) and to a lesser extent by persistency (8.4%), which together constituted 74.0% of the total variation, the remaining 26.0% being random or error effects.

On an "among-lactations-in-breed" basis, 92.1% of the total variation in lactation milk production was due to maximum initial milk yield, 0.9% to added effect of persistency, and the remaining 7.0% of the variation to random or error effects.

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OPEN SHED AND PORTABLE PENS VERSUS CONVENTIONAL HOUSING FOR YOUNG DAIRY CALVES[1]

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SUMMARY

One hundred and twenty-two female calves raised from three days of age to 16 wk., either in conventional barns, in open sheds, or in portable pens showed no advantage of one type of housing over another in growth of the calves. Exercise in a dry lot from 8 to 16 wk. was of no apparent value as compared to confinement in individual pens. Terramycin-fed calves exhibited significantly greater body weight gains ($P < 0.05$) during the first 8 wk. of the trial, during which time the antibiotic was fed, irrespective of type of housing between conventional barns and portable pens. This accelerated rate of gain did not persist after antibiotic feeding ceased and at 16 wk. of age no differences in rate of gain were evident. The mean temperature and relative humidity in the conventional barns were slightly higher throughout the 4-yr. trial than in either the open sheds or the portable pens. Calves housed in the portable pens consumed slightly more hay than those in the conventional barn, but starter and milk were similar.

The use of open-shed housing for dairy cattle has become widespread in recent years; however, very little experimental data is available on the minimum housing requirements for young calves. Research workers at Wisconsin *(6)* made an extensive study on loose housing for lactating dairy cows. These cows' respective calves were raised in individual pens located in the loose house. The calves appeared hardier when turned on pasture, although their controls, housed in conventional barns, grew faster. However, Heizer *et al. (4)* later concluded that the differences in rate of gain between the two groups were not attributable to effect of housing.

Erb *et al. (3)* reported a mortality rate of 24% for young calves housed in conventional barns as compared to 2% for those housed in open sheds. Minor frost-bite on the ears occurred in the loose-housed calves when the environmental temperature dropped to as low as −20° F. No significant differences were apparent in body weight gains, although calves housed in the open shed consumed significantly more total digestible nutrients (TDN) per 100 lb. body weight.

Alabama workers *(2)* reported excellent results with portable outside pens, in the control of coccidiosis as well as in promoting better growth. Respiratory disturbances and pneumonia were more prevalent among control calves housed in a conventional barn than among those raised in the portable outside pens. Roy *et al. (5)*, in a pasture-grazing trial with young calves, observed no ad-

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verse effects attributable to weather when calves were sheltered from prevailing winds and rain by a cluster of bales of straw located out on pasture.

Inasmuch as favorable results were observed elsewhere with open-type housing for young dairy calves, the purpose of this study was to ascertain the relative value of open-shed housing and portable outside pens as compared to conventional housing for young calves raised in the Southeast. The value of exercise for the calves was also studied.

EXPERIMENTAL PROCEDURE

The investigation, continuing over a 4-yr. period, was conducted in two separate trials.

Trial I

Seventy-four female calves of the Ayrshire, Guernsey, Holstein, and Jersey breeds were randomly assigned within breeds at three days of age either to a closed, conventional barn or to an open-shed type of housing. The conventional barn used was an 18 by 72 ft. building, with box stalls on each side of a center alley-way. The individual box stall had solid partitions between calves. A window opening inward at the top was located above each stall. The open shed was a building about 20 ft. deep, with an eastern exposure. Temporary pens, 4 by 6 ft. in size, constructed of 1 by 6 in. boards, placed 4 in. apart, were placed in clusters of four under the open shed. The calves were housed in individual pens in these two types of housing to 8 wk. of age. At that time the calves within each type of housing were further subdivided (Table 1), whereby one group of 15 calves remained in their respective individual pens to 4 mo. of age. The other 22 calves of each housing group were placed during the day on a dry lot, which was made free of vegetation by chemical treatment with C.M.U. soil sterilizer (3-*P*-chlorophenyl-1,1-dimethylurea), and returned to their individual pens at night.

TABLE 1

Average body weight gains of calves raised in different types of housing

Type of barn	Treatment	No. calves	Av. body weight gain			
			First 8 wk.		Full 16 wk.	
			Total	Daily	Total	Daily
		Trial I (1952–53)				
Closed	No exercise	15[a]	....	.....	112[b, c]	1.00
Closed	Exercise	22[a]	....	.....	114[b, c]	1.02
Open shed	No exercise	15	....	.....	113[b, c]	1.01
Open shed	Exercise	22	....	.....	112[b, c]	1.00
		Trial II (1954–55)				
Closed	Basal ration	12	51	0.91	129[e]	1.15
Closed	Basal and terramycin	12	59[d]	1.05	126[e]	1.13
Portable pen	Basal ration	12	55	0.98	133[e]	1.19
Portable pen	Basal and terramycin	12	67[d]	1.20	132[e]	1.18

[a] One calf died of pneumonia.
[b] L.S.D. for closed vs. open barn was 11.2.
[c] L.S.D. for exercise vs. no exercise was 11.4.
[d] The 8-wk. body weight gains of the antibiotic-fed calves were significantly greater ($P < 0.05$) than the controls.
[e] L.S.D. for portable pen vs. closed barn was 10.6.

A standard feeding regime prevailed, consisting of whole milk initially fed at the rate of 10% of body weight, gradually reduced to 6% of body weight, and terminated at 8 wk. A commercial calf starter, limited to a maximum of 4 lb. daily, was fed to 12 wk. of age after which a simple grain mixture was used. Alfalfa hay was fed ad lib. from 2 wk. of age.

Throughout the trial a continuous recording was made of the environmental temperature and relative humidity in both barns.

Trial II

Twelve female calves, comprising three each of the same four breeds used in Trial I, were assigned to each of four experimental groups (Table 1) in a randomized-block design, replicated by breeds. The same conventional barn was used as in the previous trial and portable pens were constructed according to specifications reported by the Alabama workers *(1)*. The calves remained in these two types of housing from three days of age throughout the 16-wk. trial.

A feeding regime similar to that in the previous trial prevailed, except that one-half of the calves in each type of housing received via the milk approximately 60 mg. of terramycin[3] daily. The milk- and terramycin-feeding were discontinued at 8 wk. of age.

RESULTS AND DISCUSSION

Mean body weight gains (Table 1) indicate no differences among calves in Trial I either for conventional as compared to open shed–housed calves, or for dry-lot exercise *vs.* no exercise. Although the average weight gain for the calves housed in the portable pens (Trial II) was slightly higher than that of the control animals, this difference was not statistically significant.

Calves in Trial II that were fed terramycin during the first 8 wk. gained 19% faster than those not getting the antibiotics. This difference was significant statistically ($P < 0.05$). However, this accelerated growth rate did not continue after the antibiotic feeding ceased; in fact, it decreased to the extent that at the end of the 16-wk. trial body weight gains were similar.

The incidence of diarrhea was insignificant throughout the two trials. Four cases of pneumonia, two of which resulted in death, developed among the 37 calves housed in the closed barn during Trial I. The environmental temperature throughout the year, in both the open shed and the portable pens, was consistently lower than in the closed barn, especially during the winter months. The average temperature for the closed barn was 67.7° F., with ranges from 24 to 102° F.; that for the open shed, 63.3° F., with ranges from 15 to 100° F., and that for the portable pens, 60.2° F., with ranges from 13 to 100° F. Apparently, the lower temperatures did not affect the health of the calves. The mean relative humidity throughout the trial was slightly higher in the closed barn (68.9% for the closed barn *vs.* 66.4% for the other two types of housing), even though sufficient windows were opened to supply ventilation. The greater dampness

[3] An antibiotic feed supplement containing 10 g. of crystalline oxytetracycline hydrochloride per pound. Supplied through the courtesy of Pfizer & Co., Inc., Terre Haute, Indiana.

may account for the four cases of pneumonia encountered among calves housed in the closed barn. The calves housed either in the open shed or in the outside portable pens developed a rougher, thicker coat than did the control animals.

Whole milk was fed according to the weight of the calf during the first 8 wk. of the trials. Thus, the faster growing calves consumed the most milk. Starter was fed free choice up to a maximum of 4 lb. daily per calf. Both starter and hay were group-fed while calves were on exercise lots in Trial I. The average feed consumption of calves in Trial II is shown (Table 2). The calves housed in the portable pens grew slightly faster during the milk-feeding period. Thus, they consumed more milk than the calves housed in the conventional barn. Starter consumption was similar for all groups, but the calves housed in the portable pens consumed 19% more hay than calves in the conventional barn.

TABLE 2

Average feed consumption of calves housed in conventional barn and portable pens

Barn	Ration	No. calves	Milk	Starter[a]	Hay	Calc. TDN per lb. gain
			(lb.)			
Conventional	Basal	12	304	190	207	2.37
Conventional	Basal and terramycin	12	308	182	205	2.29
Portable pens	Basal	12	325	179	259	2.38
Portable pens	Basal and terramycin	12	340	184	233	2.34

[a] A commercial starter was fed for the first 12 wk., after which a simple grain mixture was fed.

The calculated TDN intake per pound of gain was similar for all groups. The calves housed in the outside portable pens apparently had a stimulated appetite for hay.

No cost or labor data were collected during these studies. Obviously, it would be less expensive initially to provide either portable pens or an open shed rather than a conventional barn for young calves. Labor would be similar for caring for calves in an open shed and the conventional type of housing, except, perhaps, that cleaning out the built-up bedding pack could be done with power equipment in the open shed. Moving the portable outside pen to clean ground periodically partially would offset the labor of cleaning manure and bedding out of stalls in the conventional barn; however, labor for watering and feeding might be greater for portable outside pen–housed calves.

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RESPONSE OF YOUNG DAIRY CALVES FED A SIMPLE VERSUS A COMPLEX STARTER WITH VARIOUS KINDS OF HAY [1, 2]

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SUMMARY

Forty-seven Jersey calves were randomly assigned at four days of age to receive either a complex or a simple starter limited to 3 lb. daily per calf. Whole milk was fed in limited amounts through 35 days of age and either Coastal Bermuda or oat hay was fed from eight through 112 days in Trial I; whereas, alfalfa or crabgrass hay was fed from 15 through 112 days in Trial II. No significant differences were observed in milk, starter, or hay consumption, or in weight gains or feed efficiency between starter groups. Calves fed the Coastal Bermuda hay exhibited significantly greater weight gains than those getting the oat hay, irrespective of type of starter fed, and were more efficient in their use of TDN for increased live weight. Although alfalfa hay–fed calves consumed significantly more hay than the crabgrass hay–fed group, growth rates were similar for both. The less efficient use of TDN for gains shown by calves fed alfalfa hay is attributed to a poorer utilization of TDN from hay, which made up a larger portion of the total TDN intake for these calves.

It is economically advantageous for dairymen to take full advantage of early rumen function in dairy calves, thus permitting the feeding of large quantities of roughage and a simple grain mixture. Research workers *(6, 7)* have shown, by the early feeding of large quantities of hay in relation to concentrate, that mature type rumen function can be established in young calves, making possible successful calf-raising on limited quantities of milk and on a high-roughage, low-cost ration.

The kind and amount of concentrate needed to adequately supplement a high-roughage, limited-milk system of raising calves has not been investigated adequately. Ohio workers *(4)* found no advantage for feeding a complex 25% protein calf starter, compared to a simple 14.5% protein starter to calves receiving either alfalfa hay or an alfalfa-clover-timothy mixture with whole milk being fed through 7 wk. of age. Additional work on this problem appeared advisable especially when even more limited quantities of whole milk are fed through only 5 wk. of age *(2)*.

Even though alfalfa hay has been shown to be an excellent calf roughage *(2, 3, 4)*, many dairymen prefer to feed a grass or mixed hay to young calves.

The main purpose of this study was to determine the relative value of a simple and a more complex calf starter when calves are fed very limited amounts

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[2] This work was conducted cooperatively with the Dairy Cattle Research Branch, ARS, USDA, and the North Carolina Department of Agriculture.

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of whole milk and various kinds of hay. Secondary objectives were to compare alfalfa hay with a grass hay and to evaluate Coastal Bermuda grass hay as a roughage for young calves.

EXPERIMENTAL PROCEDURE

Trial I. This trial was conducted at the Coastal Plain Research Station, Willard, North Carolina. Twenty-four Jersey calves were randomly assigned to one of four treatment regimes within two female and four male replicates of a randomized-block design. The four treatments consisted of a simple or a complex calf starter fed in combination with field-cured oat or Coastal Bermuda grass hays.[5] The starters described (Table 1) were prepared in a commercial

TABLE 1

Grain mixtures[a] fed to calves from 2nd through 16th wk. of age

Ingredients	Complex	Simple
	(lb.)	
Corn (coarsely ground)	470	830
Wheat bran	300	.....
Oats (crimped)	500	540
Linseed oil meal (solvent process)	100	.....
Soybean oil meal (solvent process)	300	500
Cane molasses	100	100
Dried skimmilk	200	.....
Dicalcium phosphate	14	14
Ground limestone	6	6
Salt	10	10
Vitamin A and D_2 supplement	–[b]	–[b]

[a] Proximate analyses and calculated TDN *(8)* content of the complex and simple starter, respectively, were dry matter, 90.7, 91.6; crude protein, 17.8, 18.6; ether extract, 3.5, 3.6; crude fiber, 6.0, 5.8; nitrogen-free extract, 56.7, 58.0; ash, 6.7, 5.7, and TDN, 73.6, 75.9%.

[b] To supply a minimum of 10,000,000 and 4,000,000 U.S.P. units of vitamin A and D_2, respectively, per ton.

feed-mill and were the same for Trials I and II. The calves remained with their respective dams for three days after birth and were fed thereafter limited amounts of whole milk, according to a predetermined schedule, through 35 days of age *(2)*. Hay and water were fed ad libitum and the concentrate mixture up to a maximum of 3 lb. daily per calf was fed from eight through 112 days of age. The calves were housed in individual pens.

Trial II. Trial II was conducted at the Mountain Research Station, Waynesville, North Carolina. The feeding and management regime and the experimental design were similar to those outlined for Trial I, with three exceptions: *(a)* Twenty-three[6] female Jersey calves were used. *(b)* Barn-dried alfalfa and field-cured crabgrass were the hays fed. *(c)* Starter and hay were fed from 15 through 112 days of age.

[5] The oat hay was yellow-green, cut in the pre-dough stage, had no foreign material present, graded U.S. No. 2, contained 10.7% crude protein, and had an estimated TDN content *(8)* of 60.1% on the as-fed basis. The Coastal Bermuda grass hay averaged 47% color, 12.5% foreign material, graded U.S. No. 2, had a crude protein content of 13.2%, and an estimated TDN content *(8)* of 61.1%.

[6] The mean of the other calves on the same treatment was taken as the value for the missing calf, and one degree of freedom was deducted from error in the analysis of variance.

Chemical analyses for both starters and for the oat and Coastal Bermuda grass hays were carried out by standard A.O.A.C. procedures *(1)*. TDN values for these feeds were then estimated by the method of Schneider *et al. (8)*. The TDN values for the milk (19%) and the crabgrass hay (47%) were taken from Morrison *(5)*; whereas, that for the barn-dried alfalfa hay was assumed to be 56%.

The response criteria were analyzed by usual statistical methods *(9)*. Adjustment of these criteria for the appropriate seven-day observation by covariance was inappreciable; therefore, only unadjusted means and significance levels are reported. Since the variances for the same criterion in Trials I and II were not all homogeneous, the means for both trials (Table 2) were combined by weighting each mean by the reciprocal of its variance.

TABLE 2

Effect of a simple versus a complex starter and various varieties of hays on mean feed intake, growth, and efficiency of feed utilization by Jersey calves

	Starter		Hay		Standard error of a mean
	Simple	Complex			
Trial I					
			Coastal Bermuda	Oats	
Initial weight *(lb.)*	58.7	53.1	54.4	57.4	.....
Milk *(lb.)*	121	114	115	120	.....
Starter *(lb.)*	229	230	232	226	4.7
Hay *(lb.)*	104	111	104	111	5.8
TDN *(calc.)* *(lb.)*	261	257	258	260	5.1
Daily gain *(lb.)*	0.99	1.02	1.05[a]	0.96	0.025
Height gain *(in.)*	6.10	5.92	6.08	5.98	0.245
TDN above maintenance/lb gain	0.94	0.91	0.86[b]	0.99	0.032
Trial II					
			Alfalfa	Crabgrass	
Initial weight *(lb.)*	61.8	61.6	61.5	61.9	.....
Milk *(lb.)*	122	123	122	123	.....
Starter *(lb.)*	215	218	218	215	8.2
Hay *(lb.)*	130	160	168[b]	122	19.9
TDN *(calc.)* *(lb.)*	249	270	283[a]	237	8.8
Per cent total TDN from hay	26.6	29.9	32.7[a]	23.8	2.02
Daily gain *(lb.)*	0.89	0.91	0.93	0.87	0.031
Height gain *(in.)*	6.29	6.03	6.42	5.90	0.181
TDN above maintenance/lb gain	0.96	1.14	1.23[a]	0.87	0.066
Weighted means and standard errors over both trials[c]					
Starter *(lb.)*	226	227			4.1
Hay *(lb.)*	108	118			5.3
TDN *(calc.)* *(lb.)*	258	260			4.4
Daily gain *(lb.)*	0.95	0.98			0.019
Height gain *(in.)*	6.22	5.99			0.146
TDN above maintenance/lb gain	0.94	0.96			0.029

[a] $P < 0.01$.
[b] $P < 0.05$.
[c] Combined by weighting each mean by the reciprocal of its variance.

RESULTS AND DISCUSSION

In preliminary studies the finely ground grain particles and minerals present in the starters allowed sorting of the material by the calves. Both of the starters used in the trials reported had molasses added to overcome this tendency and to reduce dustiness.

Results of both trials are presented (Table 2). No interactions between kind of starter and type of hay fed were evident for the criteria presented in either trial. Therefore, only the over-all means are presented.

In Trial I no real differences between treatment groups were evident in milk, starter, hay, or calculated TDN consumption. Calves getting Coastal Bermuda grass hay gained at a faster daily rate ($P < 0.01$) and were more efficient ($P < 0.05$) in TDN above maintenance used per pound of gain than were calves fed oat hay, a common calf roughage in this area. This advantage shown by the Coastal Bermuda lends encouragement for its use in a dairy program in this southeastern region.

In Trial II, the consumption of milk and starter was similar for the various groups. Calves fed alfalfa hay consumed significantly more hay ($P < 0.05$) and calculated TDN ($P < 0.01$) than calves fed crabgrass hay.

Significant differences were found for TDN above maintenance per pound of gain between hays ($P < 0.01$), with calves on crabgrass being more efficient. The higher hay consumption by the calves fed alfalfa hay probably accounts for this difference. As seen (Table 2), a larger proportion of the calculated TDN intake for calves fed alfalfa hay ($P < 0.01$) came from the hay which may have been used less effectively than TDN from starter and milk for increase in body weight. One of the calves on alfalfa and complex starter consumed twice as much hay as any other calf in the trial and 61% of his total TDN intake was derived from the alfalfa hay.

Weighted means over both trials reveal no significant differences in feed consumption, body growth, or feed efficiency between starter groups. Therefore, in these trials, no advantage was apparent from feeding the more complex calf starter. Thus, it appeared that the calves were able to utilize these hays supplemented with a simple calf starter for normal gains at an early age even though whole-milk feeding was very limited. Whether this would hold true when lower-quality hays were fed needs further investigation.

Although the alfalfa hay–fed calves consumed significantly more hay than the crabgrass-fed calves, they grew at a similar rate. Whereas no advantage was noted in feeding alfalfa hay compared to crabgrass hay, no disadvantages were noted for feeding alfalfa as the only roughage to young calves.

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NONRETURN RATE OF ARTIFICIALLY INSEMINATED DAIRY COWS AS AFFECTED BY AGE OF SEMEN, BREED OF BULL, AND SEASON[1]

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SUMMARY

Records from 71,098 first services involving 60 bulls from six dairy breeds were obtained during 1951 and 1953. The breed of bull, age of semen, date of service, and collection number were recorded in the field on IBM mark-sense cards. Records of repeat services included the code number of the bull previously used, and the collection number, so that unsuccessful first services could be identified. It was found that: (a) Nonreturn rate decreases from five to 11 percentage units (depending on breed) for each additional day of storage of the semen. (b) Breeds, as represented in this study, have different potential nonreturn rates, and these rates are differentially affected by storage of the semen. (c) Seasonal effects on the percentage of nonreturns depend, somewhat erratically, upon the age of the semen. (d) Although the decline in fertility of stored semen is least rapid in the winter months of December, January, and February—as might be expected—it is no greater in the summer than in the spring and fall seasons. However, it may be that apparent seasonal effects are partially confounded with dairy husbandry practices associated with recommended time between calving and first service, milk pricing systems, and types of milk marketing.

Some of the controllable factors which are believed to affect nonreturns among artificially inseminated dairy cows are: *(a)* The time elapsed from the collection of the semen until its use, *(b)* the health, age, and breed of the bulls and cows, *(c)* the season of the year during which insemination is attempted, *(d)* the diluent used, and *(e)* the skill of the inseminator. Some of these factors are not measured objectively and frequently enough to produce adequate data for statistical analysis. However, the age of the semen, season of year, and breed of bull —the subjects of this paper—can be recorded readily on punch cards and thus be made available for analysis.

A number of workers have reported on the effects of the age of the semen on the percentage of nonreturns *(5, 10, 11, 14–17, 19, 20)*. They generally have concluded that nonreturn rate decreases from four to eight percentage units for each additional 24 hr. of storage, up to four days. Willett *(19)* found that the degree to which the semen was diluted affected the rate of decline in nonreturn rate with additional storage.

Schultze *et al. (15)*, in Nebraska, concluded that nonreturn rate was lowest in July and August. Erb and Waldo *(9)* presented a comprehensive review of seasonal and monthly variations in breeding efficiency under artificial insemination. They reported that in northwestern Washington the lowest breeding efficiency was obtained during the period from January through April, and the

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[1] The data for this study were provided by the Kansas Artificial Breeding Service Unit.

highest during September, October, and November. Mercier and Salisbury *(12)* found similar seasonal effects on breeding efficiency in New York.

Climatic conditions in Kansas may be more nearly typical of those in the southern half of the United States (from which few studies of nonreturn rate under artificial insemination have been reported) than they are of the northern half. One purpose of the study reported herein was to determine whether, under Kansas conditions, there are breed, season, and interaction effects relative to the storage capacity of semen produced for artificial insemination.

MATERIALS AND METHODS

Procedures typical of artificial breeding organizations were followed in collecting, evaluating, and processing the semen used in this study. The usual practice was to collect from each bull once a week. Collection time was between 8 and 10 A.M. every other day. A minimal standard of 12 million motile spermatozoa per cc. of extended semen was maintained. Some variation in the rate of extension occurred, depending on the demand for semen and on the volume and quality of the semen collected. This was particularly true before 1953 when, based on a microscopic evaluation of density, the semen was extended only to the extent of satisfying the demand. No semen which was rated as having less than 50% motility was used. Semen was extended in an egg yolk–citrate diluent, to which was added 800 units of penicillin and 800 μ of streptomycin per cc. of extender. Diluted semen was slowly cooled at 40° F., then packaged for shipment. Approximately 3 lb. of ice were required in the insulated shipping carton to insure adequate refrigeration during shipment by bus or by mail (parcel post). Semen was shipped every other day to all inseminators.

Sixty bulls distributed among the Ayrshire, Brown Swiss, Guernsey, Holstein, Jersey, and Milking Shorthorn breeds were involved in this study, but 37,628 of the 71,098 first services reported were from Holstein bulls. The period covered by this study involves the calendar years 1951 and 1953.

At the time of service, the inseminator filled out a mark-sense IBM card which included the following information: *(a)* The bull code number, collection number, and breed of bull, *(b)* the inseminator's code number, *(c)* the age of the semen in hours, *(d)* the date of the service, *(e)* whether this was a first or a repeat service, and *(f)* the code and collection number of the previous bull used, if this was a repeat service. For purposes of the present study, the enumeration data so obtained were summarized with respect to inseminator, breed of bull, month of service, age of semen in 12-hr. intervals, nonreturns within 90 days for a given classification of data (by breed, month, and age of semen), and the total number of services for that classification.

Three types of statistical analysis were employed; namely, chi-square tests and linear regression and graphic analyses. A supplementary study, using the angular transformation and analysis of variance suggested by Campbell *(5)* and by Anscombe *(4)*, was made on the Holstein data, to see if it would add information not obtained from the simpler tests listed above. It did not; therefore, only the simpler, and somewhat more logical, tests are reported herein.

Preliminary statistical studies were made to see if the inseminators were adding an important source of variation among nonreturn rates. Chi-square analyses of inseminator × bull contingency tables indicated that the inseminators did not produce an important source of variation in breeding efficiency.

RESULTS AND DISCUSSION

A. *Effects of age of semen and of breed of bull on nonreturn rate.* The nonreturn rates obtained with the six breeds after the selected ages of the semen are shown (Table 1). Table 1 indicates that the breeding efficiency of semen may

TABLE 1

Numbers of first services (T), percentage 90-day nonreturns (NR), and storage times of the semen by breeds

	Age of semen *(hr.)*					
Breed	⩽ 24	25–36	37–48	49–60	> 60	Breed totals
Ayrshire						
T	453	963	311	803	107	2,637
NR(%)	63	62	64	54	45	
Brown Swiss						
T	732	1,487	615	1,251	160	4,245
NR(%)	70	67	62	59	63	
Guernsey						
T	1,645	3,680	1,281	3,090	319	10,015
NR(%)	66	62	55	51	44	
Holstein						
T	6,977	13,448	5,052	11,099	1,052	37,628
NR(%)	71	68	65	63	56	
Jersey						
T	806	1,931	616	1,582	159	5,094
NR(%)	69	65	67	57	57	
Milking Shorthorn						
T	1,963	4,195	1,372	3,566	383	11,479
NR(%)	70	66	56	54	49	
				Total first services		71,098

decline significantly with additional storage, and that this decline may depend somehow on the breed of the bull involved. It was concluded from preliminary graphic analysis that it would be useful to mesaure the linear decline in nonreturn rate with additional hours of storage of the semen before use in insemination. Hence, a straight line was fitted by the method of least squares to the NR-storage scatter diagrams. Because the two end-classes of age of semen (shown at the top of Table 1) involved ages spread nonuniformly between 4 and 24, and 61 to 99 hr., respectively, it was decided, after some study of actual frequencies, to use 18 and 72 hr. as the respective mid-points of those open classes when computing the regression coefficients (shown in Table 2). The actual mid-points of the other time intervals (shown in Table 1) were found to be suitable for the regression analyses (summarized in Table 2).

With the large numbers of services involved in this study, all breed regression coefficients are statistically significant, and all differences between pairs of

TABLE 2

Estimated linear reductions in nonreturn rate with each additional day (24 hr.) of storage of the semen within each of six breeds of dairy cattle

Breed	Number of records	Estimated reduction in nonreturn rate for each additional day of age of semen
Holstein	37,628	5.6
Brown Swiss	4,245	6.4
Jersey	5,094	7.0
Ayrshire	2,637	7.6
Guernsey	10,015	10.5
Milking Shorthorn	11,479	11.0
Total	71,098	

regression coefficients also are statistically significant, on the assumption that the bulls used in this study are representative of their respective breeds. Although the bulls were not selected at random for the Kansas Artificial Breeding Service Unit, the methods of selecting bulls for that unit are much the same as for most other such units. Thus, these results should be representative of artificial breeding units which operate under similar conditions. It might be that a few especially fertile (and/or low fertility) bulls distorted the breed comparisons, but this seems unlikely considering the number of bulls involved. Individual bull variance within breeds currently is being given intensive study.

The rate of decline in nonreturns found in this Kansas study may be faster with additional storage than generally has been reported from regions farther north.

Over-all breed comparisons are presented (Table 2). To determine how the breeds compare after selected periods of storage (such as those shown in Table 1) breed × return-nonreturn contingency tables and chi-square tests were used to assess the observed differences shown in Table 1. The results of these analyses are given (Table 3).

The following points of interest can be noted (Table 3), which took account of the influence of sampling errors and the small sizes of samples for the older semen: *(a)* Holstein bulls never are out of the highest ranking group for any age of semen studied; *(b)* Guernsey bulls never are above the lowest ranking

TABLE 3

Groupings of breeds into statistically homogeneous nonreturn rate groups within the indicated periods of storage of the semen[a]

Storage period	Homogeneous breed groups and average nonreturn rate									
(hr.)										
≤ 24	H,B,M,J(70)			G,A,(65)						
25–36		H(68)	B(67)	M,J(66)		A,G(62)				
37–48				J,H,B(65)			M,G,A(56)			
49–60					H(63)		B,J(58)		A,M(54)	G(51)
> 60								B,J,H(57)		M,A,G(46)

[a] The average nonreturn rate of each group is shown in parentheses. Breed is indicated by the first letter of its name.

group; *(c)* Ayrshires usually are as low-ranking as the Guernseys, the 49-60 hr. storage being the only exception; *(d)* Brown Swiss semen produced almost as high a nonreturn rate as Holstein semen, provided it was not more than 48 hr. old. A similar statement holds for Jersey semen; *(e)* Milking Shorthorn semen produces nonreturn rates as high as any of the other breeds studied, if used within 24 hr. of its collection.

B. *Effect of season of year on nonreturn rate.* There was no evidence that any of the breeds differed materially in the proportions of services from one month to the next; therefore, all breeds were combined in studying monthly fluctuations in nonreturn rate. It did seem, however, that possible seasonal effects might depend partially on the time elapsed between the collection of the semen and its use. Hence, the effects of season were studied for one-, two-, and three-day-old semen. The nonreturn rates by month and by season are shown (Table 4). The month, or season, refers to the time of the first service.

The chief information contained in Table 4 is shown graphically (Figure 1). In general, it is concluded (Table 4) that July and early August are periods which are likely to be associated with lower nonreturn rates from first services. Thereafter, the nonreturn rate can be expected to rise rapidly through September. The breeding efficiency continues to rise to a peak in December, drops rapidly to a low in the middle of the winter season, or holds up well until spring, depending on whether the period of storage is one, two, or three days. With semen used within 24 hr. of its collection, there may be another (but lower) peak for nonreturn rate in April; but, this is not true for semen used more than one day after collection.

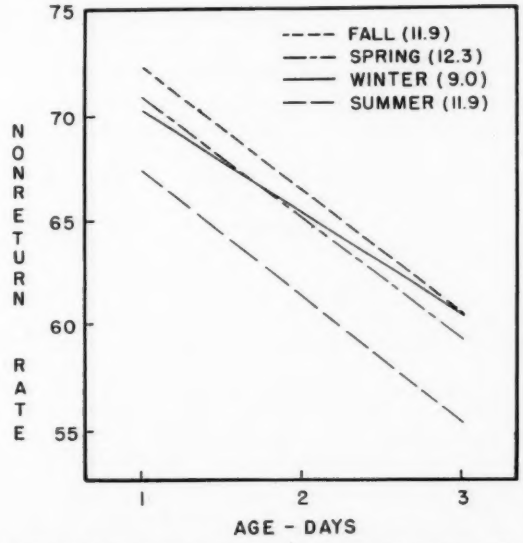

FIG. 1. Relationship between nonreturn rate and age of semen, and its dependence on season of the year.

TABLE 4

Monthly and seasonal summary of inseminations during a 2-yr. period, involving 60 bulls distributed among six breeds

	Length of storage									
	1 day			2 days			3 days			Three-day decrease in NR
Month	No.	NR	Season NR	No.	NR	Season NR	No.	NR	Season NR	
Dec.	1,498	69.4		4,440	66.3		2,956	62.6		
Jan.	1,638	67.1	68.5	4,132	66.3	65.7	2,815	58.3	59.5	(9.0)
Feb.	974	69.3		2,912	64.0		1,829	56.3		
Mar.	918	70.9		2,972	67.0		1,770	60.8		
Apr.	984	72.5	71.7	2,453	61.6	64.8	1,773	57.9	59.4	(12.3)
May	1,079	71.7		3,123	65.2		2,084	59.5		
June	814	69.5		1,638	64.7		2,065	56.8		
July	877	65.2	67.4	2,300	60.0	61.5	1,643	55.8	55.5	(11.9)
Aug.	919	67.6		2,499	60.9		1,743	53.7		
Sept.	672	75.0		1,809	67.2		1,208	59.4		
Oct.	837	73.8	72.3	1,994	66.5	66.7	1,298	60.0	60.4	(11.9)
Nov.	1,362	70.1		3,613	66.5		2,386	61.1		

If the data are studied by seasons (Table 4 and Figure 1) chi-square tests show beyond all reasonable doubt ($P < .001$) that, for each age of semen, the proportions of returns and nonreturns in 90 days are much different from season to season. For all three ages of the semen, the highest average nonreturn rate occurs in the fall season, and the lowest in the summer. It also is shown (Figure 1) that the semen declines in fertility linearly with additional storage.

The specific causes of the apparent seasonal fluctuations in nonreturn rates are not known. However, preliminary statistical study has shown that these fluctuations are not simply related to maximum, minimum, or mean monthly temperatures.

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TECHNICAL NOTES

NEW APPROACH TO THE PROBLEM OF ASSESSING ADRENAL CORTICAL ACTIVITY IN DAIRY CATTLE [1]

The problem of assessing adrenal cortical activity is well characterized by the work of Sandberg *et al. (8)*, who reported that human patients had elevated levels of plasma 17-hydroxycorticosteroids after surgery and at the same time had depressed rates of peripheral metabolism or degradation of hydrocortisone. Tyler *et al. (10)* have shown that impaired liver function was directly related to the postoperative rises in plasma levels of 17-hydroxycorticosteroids in man. This illustrates the need for a measure of adrenal cortical activity which combines the separate aspects of the level of circulating hormone and the rate of peripheral metabolism or turnover of the hormone into a single measure.

Ingbar and Freinkel *(3)* have presented a method for estimating the daily rate of thyroid hormone degradation and synthesis (D) in humans. To determine D, the following constants were derived: normal level of plasma protein-bound iodine (PBI) as a measure of the level of circulating thyroxine in its volume of distribution; fractional rate of turnover per day of extrathyroidal thyroxine *(k)*, as derived from use of tracer doses of L-thyroxine tagged with I^{131}; and thyroxine distribution space or volume (TDS). Then, D equals the product of PBI × k × TDS. This method adequately distinguished between myxedema, normal thyroid status, hyperthyroidism, and panhypopituitarism in man *(3)*, and was applied by Freinkel and Lewis *(1)* to sheep, in which it was found that shearing (and the consequent temperature effect) caused an increased rate of thyroxine degradation and synthesis.

Mixner and Robertson *(5)* have presented the data necessary to calculate all of these constants but relating to the peripheral metabolism or degradation of hydrocortisone in a dairy cow. In this study a nontracer dose of hydrocortisone was used to determine the fractional rate of turnover of hydrocortisone. Hechter *et al. (2)* have found that the chief corticosteroids in the blood of cattle are hydrocortisone and corticosterone in approximately a one to one ratio, with little or no cortisone being present. Since the procedure for the assay of 17-hydroxycorticosteroids *(7)*, as used by Mixner and Robertson *(5)*, does not measure corticosterone, it seemed interesting to apply the method of Ingbar and Freinkel *(3)* to their data to obtain an estimate of the daily secretion and degradation rate of hydrocortisone as secreted by the adrenal cortex of the cow.

The pertinent data presented by Mixner and Robertson *(5)* were as follows:

(a) Holstein cow, body weight of 666 kg.
(b) Normal plasma level of free 17-hydroxycorticosteroids, 11.7 μg. %.
(c) Fractional rate of turnover (b_2) of extraadrenal hydrocortisone per day = 0.077 (per minute) times 1,440 (minutes per day) = 110.7.
(d) Hydrocortisone distribution space or volume = 388,916 ml.

The hydrocortisone secretion or degradation rate per day would be the product of b, c, and d, or 5.04 g.

One of the postulates made in the above calculation is that the fractional rate of turnover reported by Mixner and Robertson *(5)* and obtained by infusion of 400 mg. of hydrocortisone is approximately the same as the turnover rate which would be obtained by use of tracer doses of hydrocortisone. In this regard, Petersen and Wyngaarden *(6)* and Migeon *et at. (4)* found a biological half-life of approximately 80 min. (turnover rate of 12.47 per day) for 4-C^{14}-hydrocortisone in normal humans while Petersen and Wyngaarden *(6)*, in addition found a half-life of approximately 115 min. (turnover rate of 8.66 per day), using nontracer doses of hydrocortisone in humans as assayed by the chemical method of Silber and Porter *(9)*. These tracer and nontracer methods for determining turnover rates of hydrocortisone agreed fairly well.

The volume of distribution of 58.4% of body weight for hydrocortisone, as determined by Mixner and Robertson *(4)*, is at least a rational value, being below the values generally reported for total body water and greater than those given for extracellular fluids. Although the accuracy of the estimate of daily secretion rate of hydrocortisone in this cow is unknown due to the uncertainty regarding the postulates made, it does present a new and important approach to the problem of assessing adrenal cortical functioning. It is possible that estimates of hydrocortisone secretion rates obtained by this method may not be accurate in an absolute way, yet might have great comparative or relative significance among animals in various physiological states and conditions.

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AN EXPLANATION FOR BOVINE PARTURITION EDEMA AND TREATMENT WITH BLOOD PROTEIN REPLACEMENTS

Certain species often have an edematous condition near the time of parturition. This syndrome in the bovine is most apparent in the subcutaneous tissues just anterior to, and in, the udder and occurs more frequently in first-calf heifers than in cows *(1)*. The edema starts to appear a few weeks before parturition, usually reaches a maximum near the time of parturition, and gradually disappears within a few weeks after parturition. A means of controlling parturition edema is desirable, because it is the belief of many dairymen that in severe cases the swelling may permanently damage the udder and its attachments, and that animals which are potentially the highest producers are more susceptible. Many treatments have been prescribed, including prepartum milking, but none has proven generally effective *(1)*.

Recent studies have suggested that there is a parallelism between the observable edema and the characteristic drop in the serum protein level of the blood at parturition *(3)*. The level of serum proteins in the blood of heifers tended to reach a lower level than did that of cows at parturition. This drop in the serum protein level was due chiefly to β_2- and γ_1-globulins passing from the blood to the mammary gland in the process of transferring passive immunity to the new-born calf by way of the colostrum. High-producing animals tended to transfer more of these blood proteins to the colostrum. These observations suggested that the decrease in the blood serum protein level at parturition, with a resultant decrease in osmotic pressure, was responsible for the edema.

Serum albumin is the chief regulator of osmotic pressure in the blood and on a weight basis is approximately two and one-half times as effective as globulin because of the low molecular weight of serum albumin. In four cows no rapid drop in the serum albumin level was found at parturition; however, the average level at this time (2.8 g. per 100 ml. of blood serum) was lower than that at the time when the level was considered normal (3.2 g. per 100 ml.) *(3)*. Even though the serum globulins do not exert as much osmotic pressure as serum albumin, the large quantities of β_2- and γ_1-globulins which leave the blood could cause an appreciable decrease in osmotic pressure. A decrease in globulins of 1.4 g. per 100 ml. of serum, plus a drop in albumin of 0.4 g. per 100 ml., should decrease the osmotic pressure approximately 20%.

This is a study to determine if parturition edema can be controlled by raising the blood osmotic pressure with suitable blood protein replacements.

Experimental Procedure

In initial experiments two cows were given intravenously a 6% solution of Dextran, a polymerized glucose dissolved in saline used as a blood plasma extender in humans (supplied under the trade name of Fxpandex by the Commercial Solvents Corp., Terre Haute, Indiana). Both of these animals showed some improvement by the following day (Table 1); however, in a few days the severe edema had returned. It is known that Dextran is eliminated from the

TABLE 1

Effect of intravenous injection of dextran or serum albumin on udder edema at the time of calving

Cow No.	Material injected	Amount injected	Edema rating [a]		
			Before treatment	One day after treatment	Four to six days after treatment
		(liters)			
1	6% Dextran	1.0	4	3	4
2	6% Dextran	1.5	4	3	4
3	30% Serum Albumin	1.0	4^+	2^-	1^+
4	30% Serum Albumin	1.5	4^-	2	2^-
5	30% Serum Albumin	1.7	4	1	1^+
6	30% Serum Albumin	1.2	4^+	1	0[b]

[a] Average rating of from three to six judges, on a scale of 0 to 5.
[b] Died on the fourth day.

blood and the return of the edema paralleled the expected disappearance of the Dextran *(2, 4)*. It also became apparent that to raise the osmotic pressure in the blood without introducing initially large quantities of water, more concentrated solutions were desirable.

The next solution chosen for injection was bovine serum albumin, and a 30% sterile and nonpyrogenic solution was obtained (supplied by Armour Laboratories, Chicago, Illinois). Calculations indicated that the decrease in effective osmotic pressure at parturition was equivalent to a loss of 300–400 g. of serum albumin from the blood of a 1,400-lb. animal. Thus, injections of from 300 to 500 g. of serum albumin were made per animal, depending on its weight. This was injected intravenously into the jugular vein over a period of about 3 hr.

The results (Table 1) show that following the injection of serum albumin the edema was greatly reduced and did not return. Table 2 shows the extent of the increase in the serum protein level. It should be noted that other animals may have the same or lower levels of protein in their serum at parturition and show no outward edematous condition *(3)*. This suggests that even though animals which reach the lower levels are more prone to have edema, each animal may have its own critical level below which the edema will become evident. The level of serum proteins in the blood reaches the lowest level at parturition ± two days and the data (Table 2) may not represent the lowest level actually reached. Electrophoretic patterns of the blood serum proteins after the injections showed that the serum albumin level was above normal and remained elevated [see reference *(3)* for electrophoretic analysis procedure and conditions]. Electrophoretic studies were made also on the colostrum after parturition. The patterns indicated that more serum albumin was present in the colostrum than normally would be expected; however, the increase was insignificant relative to the amount injected.

The observable physiological responses to the injection of serum albumin varied. It was noted

TABLE 2

Effect of intravenous injections of serum albumin on the blood serum protein levels of parturient cows

Cow No.	Before treatment	One or two days after treatment	One week after treatment
	(g. protein/100 ml. blood serum)		
3	6.41	6.59	6.75
4	5.82	6.58	—
5	5.71	7.52	7.50
6	6.90	8.72	(Dead)

that the stalls of all treated animals were wet the following day, indicating a large excretion of water. One animal was noted giving off water vapor via the skin and also defecated a colorless fluid. Only one animal (No. 6) had a previous parturition. By 12 hr. after injection, this animal showed an obvious loss of weight, refused food and water, and had developed respiratory difficulties. It is noted (Table 2) that the serum protein level went to an extremely high level by the next day. Examination by veterinarians at this time indicated that large amounts of fluid had accumulated in the lungs. The increasing respiration difficulties caused the rupture of some of the air sacs. This animal was found dead 84 hr. after injection. At autopsy these findings were confirmed. This was the only cow used and the only animal having respiratory difficulties; thus it is not known if parity had any effect on the reaction to the serum albumin.

This investigation supports the hypothesis that the edema often observed at parturition in the bovine is caused by the decreased blood serum protein level and may be alleviated by injections of suitable solutions, such as serum albumin, to raise the blood osmotic pressure. However, the serum albumin solution used is not available at present commercially and if it were the amount required would probably make the cost prohibitive in practical terms. Because a small number of animals were used, and one

of them died, it is readily apparent that the results of this report are of a preliminary nature and by no means constitute a recommendation for practical use.[1] This elucidation of the physiological cause of parturition edema and its apparent cure suggests the importance of futher studies with serum albumin and other more readily available blood proteins and protein substitutes.

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[1] Since the measures of edema are subjective, it is necessary to use only the most severe cases to show an unquestionable reduction in the edema. The infrequency in occurrence of severe cases which can be subjected to this treatment, the cost of approximately $200 per cow for the albumin solution, and the unquestionable reduction in edema seem to justify publication in spite of the limited data. These data were collected over a 4-yr. period.

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EFFECT OF TWO SERVICES DURING ESTRUS ON THE CONCEPTION RATE OF DAIRY COWS[1]

It has been rather definitely established that the highest conception rates in dairy cows are obtained by natural or artificial service toward the end of the standing heat period *(7)*. However, a demonstrated variability in the time of ovulation *(2, 7)* may result in failure to conceive with late-ovulating cows, because of possible exhaustion of spermatozoa. Early reports *(1, 3)* indicated a striking advantage in double insemination. With limited data, Trimberger and Davis *(8)* declared double services to be impractical, after finding only a slight increase (82.5 to 84.0%) in nonreturns to first service of cows bred in the middle of estrus only, or rebred in 24 hr. To test the effect upon conception rate of double services during a single heat period, this project was initiated at the New Jersey Agricultural Experiment Station several years ago.

Beginning on January 1, 1953, a random sample of heifers and cows of the Holstein and Guernsey breeds of the Station herd were artificially inseminated twice during the same heat period. The first insemination was performed towards the end of standing heat, at a time when the animal was generally considered to be in the proper breeding condition within the scope of accepted managerial practices. A second insemination of randomly selected animals took place between 12 and 20 hr. later, when the animals had ordinarily passed the stage of standing heat for several hours.

Data including a total of 1,028 single and double inseminations with freshly extended or frozen semen from a large number of sires became available for analysis. Fresh semen was processed, using standard procedures, by the Experiment Station and the Sussex County Cooperative Breeding Association, Inc. Most frozen semen used in the trial was processed, frozen, and stored at − 79° C. by the methods outlined by Mixner *(4)*. Similar semen in the same storage bank has maintained its fertilizing capacity for a period which has now reached 2 yr. *(5)*. A few selected frozen semen samples of acceptable quality from outside sources were used and included in the study. The number of services, single or double, required for conception, covering all data, averaged 1.87, a value which compared favorably with estimates from various investigators as listed by Morrison and Erb *(6)*.

Data from two breeds, Holstein and Guernsey, were compiled, analyzed separately, and combined. Because an increase in the number of cases should be of greater importance than breed effects, only the results of the combined data are presented (Table 1). The conception rates were determined from ratios of all pregnancies to all services in cows which ultimately become pregnant. Results of analyses covering nonreturn to first service and conceptions in subsequent heat periods showed no systematic trends of significance. Type of service and type of semen appeared to be random for each cow at each heat period, and there was no evidence of a bias caused by the mixed pattern of service.

With fresh semen, double service increased the conception rate from 53.7 to 56.1%; with frozen semen a corresponding increase from 50.4 to 52.7% was found. In both cases, however, sta-

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tistical analysis (chi-square) did not show these differences to be significant. The small difference in conception rate between fresh and frozen semen indicated that frozen semen, employed to take advantage of more selected matings, has been used in the Station herd without decreasing conception rates.

Owing to the relatively small increase in conception rate and its lack of significance, double services of relatively short interval within the same estrus can not be recommended on a herd-wide basis. It is suggested that projected studies of this type should be limited to cows with lower breeding efficiency, i.e., cows which require three or more services for conception and which have a history of low fertility. Moreover, the intervals between double services should be varied under more controlled conditions.

TABLE 1

Conception rates from single or double services with fresh or frozen semen

Type of service	Services	Conceptions	Av. services per conception	Conception rate
		(No.)		(%)
Fresh semen				
Single	633	340	1.86	53.7
Double	173	97	1.78	56.1
Sum	806	437	1.84	54.2
Frozen semen				
Single	129	65	1.98	50.4
Double	93	49	1.90	52.7
Sum	222	114	1.95	51.4
Grand total	1028	551	1.87	53.6

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LOSS OF CERTAIN ACIDIC CARBONYL COMPOUNDS DURING THE FROZEN-STORAGE OF CHEESE [1]

In cheese research, it has become a common practice to place cheese in frozen-storage after specific periods of curing. The cheese may be kept in the frozen state for several months to permit detailed analysis. Usually, results obtained in this manner are representative of the cheese at the time it was placed in storage. In this regard, the changes in the free amino acid and free fatty acid contents of Romano, Provolone, Swiss, and Cheddar cheese were found by the authors to be negligible and within the accuracy of the method used during a storage period of 6 mo. at − 10° F.

However, recent observations have indicated that certain acidic-carbonyl constituents of cheese are altered during storage at − 10° F. This was first observed in the analysis of ten Blue cheeses which had been stored at −10° F. for 1 yr. Beta-keto acids which have been reported as normally present in Blue cheese *(1)* were completely absent in these cheeses. This suggested either that all ten cheeses were abnormal or that some changes had occurred during the storage period.

More conclusive evidence of changes in acidic carbonyl compounds during cold storage was obtained in respect to Cheddar and Romano Cheeses. Samples of three Cheddar cheeses, all of which contained relatively large amounts of the beta-keto acid, alpha-acetolactic acid, and smaller amounts of oxalacetic acid, were placed in storage at − 10° F. after 1.5 mo. of ripening. Each sample constituted a 2- to 3-lb. cut of cheese stored in a Cryovac bag from which the air had been evacuated. After 1 yr. of storage, the alpha-acetolactic acid in two of these cheeses had completely disappeared, and was present in minor concentrations only in the third cheese. Oxalacetic acid had disappeared from all three

[1] Technical Note No. 3–58: Ohio Agricultural Experiment Station Journal Article.

cheeses. There was no apparent change in the concentrations of the other keto acid constituents.

A two-month-old Romano cheese, which contained the beta-keto acids, alpha-acetolactic acid, oxalacetic acid, and acetoacetic acid, was analyzed at the end of 1, 2, and 3 mo. of storage at 10° F. The sample had been ground and packed in a glass container prior to freezing. No apparent change could be observed in the amounts of keto acids in the Romano cheese after 1 mo. of storage. At the end of 2 mo. the oxalacetic acid was missing from the cheese and, after 3 mo. of storage, oxalacetic acid, acetoacetic acid, and alpha-acetolactic acid were not detected.

The exact chemical nature of the change in the beta-keto acids has not been determined. However, an increase in neutral carbonyl compounds in the cheese, together with the known instability of the carbonyl group in beta-keto acids, suggests a decarboxylation reaction. Based on these observations, the recommendation is made that caution should be exercised in analyzing for beta-keto acids and neutral carbonyl compounds in cheese kept in frozen-storage. The exact time that each given variety may be stored in the frozen state before analysis must be established experimentally

Since the beta-keto acids are precursors of flavor components, interpretation in respect to flavor of frozen cheese needs to be considered. In Cheddar cheese there does appear to be some change in flavor which may be associated with changes in the beta-keto acids. However, in Romano cheese no marked alteration in flavor could be observed. This does not exclude the possibility that subtle changes in flavor do occur during frozen storage.

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REFERENCE

(1) BASSETT, E. W., AND HARPER, W. J. Acidic and Neutral Carbonyl Compounds in Various Cheese Varieties. *J. Dairy Sci.*, **39**: 918. 1956.

A MELTING TEST FOR PASTEURIZED PROCESS CHEESE SPREADS

Various unpublished methods for measuring the melting properties of process cheese products are used in commercial laboratories. They usually require the rather difficult estimate of areas covered by the cheese before and after some prescribed heat treatment. The logical method for process cheese described by Arnott, Morris, and Combs,[1] published after this study was completed, would be difficult to apply to the less stable pasteurized process cheese spread. Methods which expose the cheese to air during melting tend to cause film formation on the surface and uneven flowing of the melting spread. The melting test described here eliminates film formation and controls direction of flow to permit accurate measurement.

A Pyrex glass tube, 30-mm. i.d. and 250 mm. long, is used to hold the spread during the melting test. One end of this melting tube is closed with a rubber stopper perforated by a 1-mm. i.d. glass tube to act as a vent; a reference line is etched on the glass 27.5 mm. from the opposite open end of the melting tube. This end of the tube is also closed with a rubber stopper after the sample is measured into the tube.

During the test the melting tube is placed on a rack which holds it in a horizontal position in the oven. This rack can be tilted so that the flow of hot cheese can be stopped instantly for measurement. With this tilt-control rack, several tests can be made simultaneously.

The sample to be tested is tempered to 40° F. and a measured amount, weighing 15 ± 0.2 g., is forced into the open end of the melting tube to form a cylinder of spread 30 mm. in diameter and 20 mm. long. This cylinder of spread is adjusted in the melting tube with the rubber stopper until the stopper is seated and the leading surface of the cheese is aligned with the reference line etched on the glass.

The melting tube containing the spread is tempered for 30 min. at 40° F. in a vertical position, spread end down. It is then placed in a horizontal position on the tilt-control rack in a forced-draft oven at 110° C. After 6 min. of heating, the rack holding the tube is tilted to stop the flow of spread while the distance of flow from the reference line to the leading edge is quickly measured. The tube and rack are replaced in the oven for an additional 2 min. and measurements taken again. The total distance in millimeters covered by the spread in $6 + 2$ min. of heating is called "cheese-flow."

The brief interruption of heating is advisable to measure the flow of spread which might run from one end of the tube to the other in less than 8 min. The length of the heating period described here can be varied to suit the particular product being tested.

Three tubes containing melting cheese are shown (Figure 1) on the tilt-control rack.

Cheese-flow values of 100 samples of spread made from Cheddar and Dariworld cheese were correlated with spreadability scores of the same samples determined by organoleptic examination. The melting-test values and the opinions

[1] Arnott, D. R., Morris, H. A., and Combs, W. B. Effect of Certain Chemical Factors on the Melting Quality of Process Cheese. *J. Dairy Sci.*, **40**:957. 1957.

of the judges were closely associated as indicated by a highly significant correlation coefficient of 0.8771.

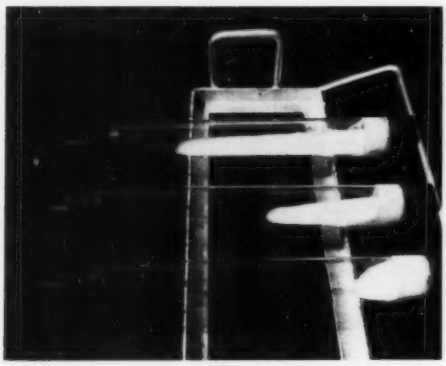

FIG. 1. Melting tubes arranged horizontally on the tilt-control rack. The three tubes show spreads possessing differing melting properties.

Spreads having cheese-flow values ranging from 100–140 mm. were considered by the judges to possess the most desirable consistency at room temperature. Spreads with cheese-flow values ranging from 80–160 mm. were acceptable.

The variability of cheese-flow values among replicates of three batches of cheese spreads is shown (Table 1). Two were classified as desirable; the third was unacceptable. The standard deviations of these replicate tests varied with the samples; the replicate tests of the sample with the lowest cheese-flow values were least variable. However, the coefficients of variation from the three series of tests were very similar, ranging from 5.0 to 6.5%.

The cheese-flow values appear to be a satisfactory measure of melting properties of spreads. The test is applicable with comparable precision to samples of varied melting properties. It eliminates dried surfaces and film formation and it guides the direction of flow of the melting spread so that a simple linear measurement expresses the effect of the melting treatment.

TABLE 1

Variability of melting test using three samples of cheese spread

Replicate	Cheese flow		
	Sample 1	Sample 2	Sample 3
(No.)	*(mm.)*		
1	110	119	71
2	105	128	75
3	99	114	66
4	114	129	70
5	117	111	74
6	105	123	67
7	98	118	66
8	109	122	71
9	118		
10	104		
Mean	107.9	120.5	70.0
Standard deviation	7.0	6.3	3.5
Coefficient of variation	6.5%	5.2%	5.0%

ACKNOWLEDGMENTS

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METHOD FOR THE RAPID ESTIMATION OF UNDENATURED WHEY PROTEINS IN NONFAT DRY MILK

Studies of the use of nonfat dry milk in baking, reported by Harland and Ashworth *(4)* and by Larsen *et al. (8)*, have shown that better dough and loaf quality is obtained if the milk has been heated sufficiently to denature whey proteins. A fast method of estimating undenatured whey protein would, therefore, be useful to the baking industry.

Many methods have been described for the estimation of undenatured whey protein. Rowland *(9)* observed that denatured whey protein would precipitate with casein in acetate–acetic acid buffer, and he used the decrease in noncasein nitrogen observed after heating as a measure of whey-protein nitrogen. Harland and Ashworth *(4)* precipitated casein and denatured whey protein with saturated sodium chloride and determined the remaining whey protein turbidimetrically. Colorimetric methods *(6, 10)* based on the biuret reaction also have been described.

Stone and Holmes *(11)* removed casein and denatured whey protein by the method of Harland and Ashworth *(4)*, precipitated the remaining whey protein with zinc hydroxide, and estimated whey-protein nitrogen by a Kjeldahl digestion, followed by direct nesslerization.

Anderson and Bell *(1)* precipitated the casein and denatured whey or serum proteins, using dilute acetic acid, and estimated the undenatured whey protein in the filtrate by adding

phosphotungstic acid, centrifuging, and measuring the volume of the precipitate.

This paper describes a spot-color test which fills the need for a rapid, reliable method of estimating undenatured whey protein and also estimates the baking quality of nonfat dry milk.

MATERIALS AND METHODS

One gram of the sample of nonfat dry milk to be tested is weighed out on a torsion balance and is reconstituted in 10 ml. of water. Then 10 ml. of 1% acetic acid is added and the mixture is stirred and allowed to stand for a few minutes until the precipitated proteins settle. Approximately 0.05 ml. of the supernatant is applied to a piece of Whatman No. 2 filter paper or similar fine-textured paper, which is supported on glass rods to keep it out of contact with the bench surface. The volume of solution applied is not critical and affects chiefly the size of the spot formed. To the eye, equal volumes of filtrates containing different amounts of undenatured whey protein give approximately the same spot size. The spot is allowed to spread for 2 or 3 min. until its maximum size is reached. After all the samples are applied to the paper, it is dried for 5 min. in an oven at 100°.

Staining of the spots is carried out according to the method described by Durrum *(3)*. In this method, the paper is immersed for 5 min. in a solution of 0.1% bromphenol blue in 95% ethanol saturated with mercuric chloride. The paper is then rinsed in running tap water for 5–10 min., or until the excess dye is washed out of the paper, leaving only the stained protein spots. (The spots slowly fade with washing, so that a definite time should be used if successive determinations are to be compared.) The paper is blotted on a towel to remove excess water and dried in an oven at 100° C. The whey-protein nitrogen is estimated by comparing the color of the spots with spots prepared from samples of known whey-protein nitrogen content by the same method. The standard spots are stable indefinitely.

For standards, and to check the results obtained by this method, undenatured whey protein was determined by a micro-Kjeldahl procedure, using the Gunning-Arnold digestion, followed by direct nesslerization *(7)*. Whey-protein nitrogen was considered to be the fraction soluble in 0.5% acetic acid and insoluble in 1% tungstic acid. This corresponds to the whey-protein fraction as determined by Anderson *(1)*, and gives higher values than those obtained by the Harland-Ashworth method.

RESULTS AND DISCUSSION

In analytical procedures for determining undenatured whey-protein nitrogen, the first step is to precipitate casein and denatured whey proteins either with dilute acid or with saturated sodium chloride. The latter procedure requires more work and careful temperature control. The use of saturated sodium chloride gives slightly different results, but no one has ever demonstrated any advantage in using this method over acid precipitation in undenatured whey protein determination. Therefore, the much simpler precipitation with dilute acetic acid was used in the present method.

To check actual values of whey-protein nitrogen against spot-test results, nitrogen was determined in the fraction soluble in 0.5% acetic acid and in that soluble in 1.0% tungstic acid by a micro-Kjeldahl procedure (7). Precipitation with 0.5% acetic acid removes casein and denatured whey proteins; tungstic acid in addition removes remaining whey proteins, proteoses, and peptones *(5)*. The difference between the two values is undenatured whey protein plus proteose-peptone nitrogen.

In the spot-test procedure, the casein and denatured whey proteins are precipitated by 0.5% acetic acid. When the supernatant liquid is applied to a piece of filter paper, the soluble proteins diffuse out to form a uniform spot. Any insoluble material which is transferred with the pipet when the spot is prepared remains at the point of application. This fact eliminates the need for filtration.

The staining technique used *(3)* was selected for its speed and simplicity. It is not as suitable for quantitative work as some others *(2)*, but it is entirely satisfactory for the purposes of this method.

Seventeen samples were analyzed for undenatured whey-protein nitrogen and the results were compared with those obtained by the spot test. These results are shown (Table 1). Table 2 shows the results obtained when the spot test was run on 20 samples of nonfat dry milk rated as being of good, fair, or poor baking quality in actual baking tests. Figure 1 shows some typical spot-test results.

Samples with very low whey protein contents and good baking quality give faint spots with indistinct boundaries. As the whey-protein content rises, the stained spots show sharp boundaries and a uniform blue color proportion to the whey-protein nitrogen. Small dark areas or rings are present in some spots and represent precipitated proteins which did not migrate from the point of application and are not considered in estimating the color. Whey-protein nitrogen can be roughly estimated by this technique. Nonfat dry milk samples of good baking quality gave faint spots without dark edges and, on this basis, they may be readily distinguished from samples of fair or poor baking quality.

This method is well suited to rapid, routine screening of the baking quality of nonfat dry milk samples. It gives results faster, is sufficiently accurate for the purpose for which it is designed, and is simple enough to be run by personnel with little technical training.

TABLE 1

Results of the spot test compared with undenatured whey protein nitrogen values

Sample	Undenatured whey protein N		
	Kjeldahl	Harland-Ashworth[a]	Spot test[b]
	—(mg/g)—		
x–2	2.0	....	±
516	2.1	....	±
44–10	2.2	....	±
7214	2.5	....	±
6628	2.5	....	±
33–7	2.6	....	±
3	3.0	1.1	±
8	3.4	1.6	±
5 (ADMI high heat std.)	3.6	1.3	±
9101	4.1	....	+
7	4.8	2.4	+
2	5.9	2.1	+
6	6.3	3.8	++
x–1	6.9	....	+++
7216	7.8	....	+++
1	10.1	5.0	++++
4 (ADMI low heat std.)	11.1	7.3	++++

[a] Data from laboratories providing samples.

[b] ± faint with indistinct edges; +, ++, +++, ++++, distinct edges, low to high color intensity.

TABLE 2

Spot test results in relation to baking quality

Sample	Actual baking quality	Spot test results	
		Spot test[a]	Estimated baking quality
3	Good	±	Good
5	Good	±	Good
8	Good	±	Good
B4	Good	±	Good
C3	Good	±	Good
C4	Good	±	Good
D3	Good	±	Good
2	Fair	+	Fair
7	Fair	+	Fair
D1	Fair	++	Fair or poor
D2	Fair	+	Fair
D5	Fair	+	Fair
D6	Fair	++	Fair or poor
1	Poor	++++	Poor
6	Poor	++	Fair or poor
4	Poor	++++	Poor
A2	Poor	+++	Poor
B2	Poor	+	Fair
C1	Poor	++	Fair or poor
D4	Poor	++	Fair or poor

[a] See Table 1.

SUMMARY

A simple spot-color test is described for use with nonfat dry milk, to estimate the amount of undenatured protein present and the baking quality. This method consists of precipitating casein and denatured whey proteins with 0.5% acetic acid and spotting the supernatant on filter paper. The paper is dried, stained with 0.1% bromphenol blue in ethanol saturated with mercuric chloride, washed with water, and dried. The intensity of the blue stain is used to estimate undenatured whey protein. The undenatured whey protein content of 17 samples was estimated by this technique. Samples of good baking quality and low undenatured whey-protein contents give pale spots, with characteristic diffuse edges. Results from 20 samples of nonfat dry milk show that this method is also satisfactory for estimating baking quality.

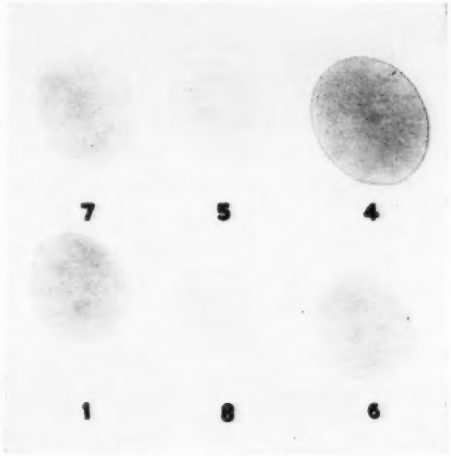

FIG. 1. Results of spot tests run with samples of known baking quality. The identification numbers refer to Table 1. Variations in spot size result from variations in the amount of sample. These photographs are of the back side of the paper to which the samples were applied.

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ACKOWLEDGMENTS

Eighteen samples of known baking quality were obtained from the Dairy Products Section of the Eastern Utilization Research and Development Division, United States Department of Agriculture. The American Dry Milk Institute provided two standard samples, one being low heat and the other high heat. We gratefully acknowledge discussions with Calvin Golumbic.

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A PSYCHROPHILIC STRAIN RELATIVELY RESISTANT TO HYPOCHLORITE-TYPE SANITIZERS[1]

Hypochlorite-type sanitizers are often used to treat washwater for butter and cottage cheese, as a precaution against bacterial contamination of the so-called psychrophilic type.

Generally, hypochlorites are considered effective against psychrophilic bacteria. However, during a study of sanitizers, a strain of *Pseudomonas fluorescens* was encountered which proved to be relatively resistant to sanitizers of all types, including hypochlorites.

Figure 1 illustrates the concentration of hypochlorite necessary to get satisfactory destruction of this bacterium in 30 sec. by the Weber and Black method.[2] For comparison, the figure includes the effective concentration of hypochlorite against an ordinary psychrophilic bacterium represented by *Pseudomonas fragi.* The resistant species of *P. fluorscens* was about five times as resistant to hypochlorite as the species of *P. fragi.*

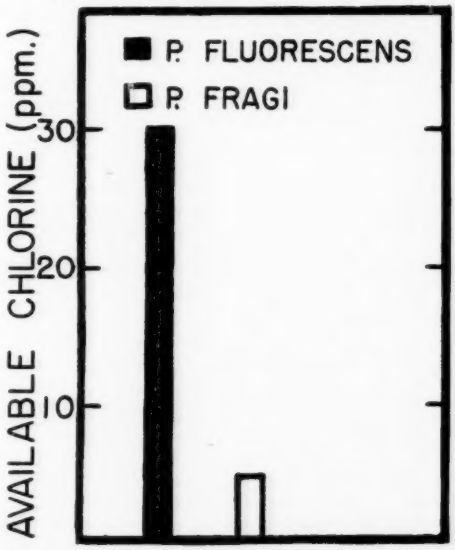

Fig. 1. Concentration of available chlorine (pH 8) necessary for satisfactory kill in 30 sec. of strains of *Pseudomonas fluorescens* and *Pseudomonas fragi.*

A survey was conducted to determine the frequency of hypochlorite resistance among psychrophilic bacteria. Twelve additional strains of bacteria of the genera *Pseudomonas, Flavobacterium,* and *Achromobacter* were included. All of these bacteria showed a low resistance to hypochlorite similar to that of the strain of *P. fragi.*

These results indicate that resistance to hypochlorites is an exception among psychrophilic bacteria. However, the fact that relatively high resistance to hypochlorites does occur among psychrophilic bacteria may well be kept in mind, if defects attributable to such bacteria persist where treated washwater is used.

ACKNOWLEDGMENT

The author expresses his appreciation to Dr. F. E. Nelson, Iowa State College, for supplying some of the cultures.

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[1] Technical Note 16:57, Department of Dairy Technology, The Ohio State University.

[2] Weber, G. R., and Black, L. A. Laboratory Procedure for Evaluating Practical Performance of Quaternary Ammonium and Other Germicides Proposed for Sanitary Food Utensils. *Am. J. Pub. Health*, **38**:1405. 1948.

VOLUME 41 July, 1958 NUMBER 7

JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

CONDENSED AND DRIED MILKS; BY-PRODUCTS

233. Dried milk product and method of making same. D. D. PEEBLES. U. S. Patent 2,835,586. 16 Claims. May 20, 1958. Offic. Gaz. U. S. Pat. Office, **730**, 3: 699. 1958.

A system is described of aggregating spray-dried milk to form porous aggregates which are characterized by high wettability and dispersibility in water to form reconstituted milk. The aggregates formed have a particle size of greater than 74 μ. R. Whitaker

234. Food pellet and method of manufacture. J. E. HIGGINS AND O. R. NEWKIRK. U. S. Patent 2,835,583. 7 claims. 1958. Offic. Gaz. U. S. Pat. Office, **730**, 3: 698. May 20, 1958.

A method of pelletizing dried whey consists of adding flour, thoroughly mixing to coat the lactose crystals, moistening the dry mix and, finally, forming pellets. R. Whitaker

235. Edible composition of vegetable oil emulsion and butter fat emulsion convertible by aeration into a stiff mass. H. M. LEVIN. U. S. Patent 2,836,497. 9 Claims. May 27, 1958. Offic. Gaz. U. S. Pat. Office, **730**, 4: 958. 1958.

A mixture of an oil-in-water emulsion is suitable for forming a foam on being released from a gas pressure-packed container. The fat is a mixture of stabilized vegetable oil and butterfat. Milk solids may be used in excess of that required for stabilization of the emulsion. R. Whitaker

DAIRY CHEMISTRY

236. Phosphatase reactivation in dairy products. T. L. EDDLEMAN AND F. G. BABEL, Purdue Univ., Lafayette, Indiana. J. Milk and Food Technol., **21**, 5: 126. 1958.

Phosphatase was determined on samples of raw skimmilk, whole milk, and cream, which were then heated over a range of 167 to 284° F. up to 15 sec. The samples were stored at 41 and 86° F. in the presence of chloroform. Phosphatase tests were negative on all samples immediately after heat treatment. In all products held at 86° F. phosphatase activity indicated underpasteurization, when held for 24 hr. Samples of milk gave negative phosphatase tests when heated to 145° F. for 30 min. and did not give a positive test after subsequent heating to higher temperatures and storage known to reactivate phosphatase in raw milk. Samples of milk containing reactivated phosphatase did not show phosphatase activity when subsequent heating to high temperatures also failed to activate the enzyme. Harry H. Weiser

237. Interrelationships of the concentration of sodium, potassium, lactose, and water in milk. J. A. F. ROOK AND M. WOOD. Nature, **181**, 4618: 1284. 1958.

A study was made of the interrelationships of these four constituents of milk by the technique of multiple regression. The partial regression coefficients of Na on K and lactose (L) are highly significant. The corresponding analysis of K on Na was highly significant, but not for L. The coefficient of L on the Na was highly significant, but nonsignificant for K. The water secreted in milk can be represented as a two-phase system—the Na-L phase where K is absent and Na and L vary inversely, and the Na-K phase where L is absent and the Na and K vary inversely. R. Whitaker

238. Rapid determination of casein in milk. J. JAKUBOWSKI, Z. SIENKIEWICZ, AND E. NOWAK. Nature, **181**, 4618: 1277. 1958.

Details are given for a method of determining casein by means of a formula involving: the acidity of the milk expressed in Soxhlet-Henkel (S-H) degrees; the S-H acidity of the whey, prepared by action of rennet on the milk; a constant given by the authors and the coefficient resulting from the dilution of the milk with the rennet solutions. Any protein hydrolysis in the milk does not significantly influence the results of this method. R. Whitaker

239. Method of and composition for preserving milk and milk products. B. HEINEMANN.

U. S. Patent 2,837,459. 2 Claims. June 3, 1958. Offic. Gaz. U. S. Pat. Office, **731**, 1: 176. 1958.

A material for reserving composite samples of milk for subsequent testing for fat, total solids, and bacteria count consists of mercuric chloride and a milk-soluble oxidizing compound, such as potassium chlorate, potassium dichromate, potassium permanganate, potassium iodate, ceric sulphate, or ammonium nitratocerate. R. Whitaker

DAIRY ENGINEERING

240. Method and apparatus for regulating the fat content of certrifugally separated cream. F. V. Haglund, Johanneshov. U. S. Patent 2,837,271. 6 Claims. June 3, 1958. Offic. Gaz. U. S. Pat. Office, **731**, 1:130. 1958.

The butterfat content of cream leaving a separator is regulated by increasing or decreasing the proportion of skimmilk to cream. The electrical resistance offered by the skimmilk and cream, when a current is passed through each as they leave the separator, is used as a basis for altering the proportion of skimmilk to cream. R. Whitaker

NUTRITIVE VALUE OF DAIRY PRODUCTS

241. Production of convulsions in hamsters by high butterfat intake. R. L. Swank and R. Engel. Nature, **181**, 4617. 1958.

Convulsions and, later, death occurred frequently when hamsters received diets containing about half the calories in the form of butterfat. Other hamsters fed the equivalent fat in the form of sesame oil did not have convulsions. The authors believe the effect is due to circulatory changes in the animals. R. Whitaker

242. Essential fatty acids and idiopathic hypercalcemia of infancy. A. T. James, J. Webb, T. Stapleton, and W. B. MacDonald, N.I.M.R., and St. Mary's Hospital Med. Sch., London. Lancet, **7019**, 1: 502. 1958.

Unsaturated fatty acids of cow's milk were determined before and after drying and after storage by use of gas-liquid chromatography which measures the sum of linoleic and linolenic acids. Analyses are reported also for human milk and samples of commercial processed milks.

There was no loss of linoleic and linolenic acids on roller-drying of cow's milk. There were losses, however, on storage at 3 and 6 mo. both at room temperature and at 37° C. The fat of human milk 3 mo. after the start of lactation contained 4.4% linoleic acid; fresh cow's milk, 3.2%. A sample of National Dried Milk contained 4.1%, and one brand of evaporated milk, 4.4%.

Three infants who had well-established hypercalcemia were studied, to determine the relationship between this condition and a deficiency in essential fatty acids in blood. Data are reported for combined linoleic and linolenic, and for arachidonic, acids. Blood levels were similar to those found in three normal infants. The hypercalcemic infants alternately were fed supplementally with and without cottonseed oil. The transient fall of serum calcium during the feeding period was believed to be due to increased excretion of calcium in the feces.

It is concluded that an essential fatty-acid deficiency is not a cause of idiopathic hypercalcemia or present in infants having this disease. Frank E. Rice

243. Essential fatty acids and idiopathic hypercalcemia of infancy. H. M. Sinclair, Oxford, England. Letter to the Editor, Lancet, **7021**, 1: 639. 1958.

This author disagrees with the conclusions reached by James *et al.* (see abstract above) and points out that the James data just as conclusively point to the low level of dietary essential fatty acids as a causative factor in hypercalcemia. Almost all the infants under the author's own observation who developed the disease had been fed dry cow's milk and not human milk or evaporated milk. A loss of essential fatty acids takes place on storage of dry milk and not on storage of milk in the liquid form.

Experiments with rats have shown that a deficiency of essential fatty acids produces an increased sensitivity to vitamin D, which may be a more direct causative factor in hypercalcemia. The higher level of calcium in a cow's-milk formula as compared with human milk is also a contributing factor.

This author questions the data reported by the James group indicating that the three hypercalcemic infants showed no lower levels of essential fatty acids than the three normal infants in blood samples. The data are questioned on the grounds that the gas-chromatography method does not exclude nonessential unsaturated fatty acids. Frank E. Rice

244. Reflections on infantile gastroenteritis and its treatment. S. Levin, Johannesburg, South Africa. J. Pediatrics, **52**, 2: 227. 1958.

This is a comprehensive review of theories, old and discredited, as well as modern, regarding the gastroenteritis syndrome in babies, the various etiologic factors and manifestations, and methods of treatment. One section is devoted to feeding, which deals almost entirely with cow's milk and its main constituents.

Until a very few years ago, it had been accepted that milk, and particularly milk fat, should be withdrawn from the diet as long

as diarrhea and other symptoms persist. Controlled clinical observations now have revealed that feeding in diarrhea should not differ from the feeding of healthy infants. The withdrawal of milk, or milk fat, or even the extreme dilution of milk induces partial starvation to a degree that weakens the infant's ability to deal with the cause of the attack.

Milk fat has been a favorite target as a causative factor in diarrhea, and some standard works today still point to the menace of fat. Evidence has been accumulating that fat is not especially harmful to infants either in health or disease. As to digestibility and assimilability, it does not differ from the fat of human milk. The replacement of whole milk with skimmilk, if prolonged, may cause a persistence of looseness of stools.

Electrolyte solutions administered orally or by infusion are useful, where there is severe diarrhea and a correlated need for water replacement. Such solutions should be used as an adjunct to milk rather than as a substitute. The substitution of clear liquids for milk is indicated temporarily where vomiting is severe, and there is concurrent danger of aspiration of vomited milk.

In cases of mild diarrhea, the author mainly advocates doing whatever is necessary for the curbing of distracted relatives.

Frank E. Rice

245. Essential fatty acids in infant feeding. A. E. HANSEN, Univ. of Texas Sch. of Med., Galveston. J. Am. Dietetic Assoc., **34**, 3: 239. 1958.

The author discusses the differences between human milk and the infant formula prepared from cow's milk. He rejects the possibility that from a nutritional point of view human milk may better provide vitamins, proteins, minerals, or carbohydrates, or that antibiodies, enzymes, or pigments may have any significance. Breast feeding may have a role in strengthening the emotional ties between mother and child.

The relative merits of the two types of feeding, however, should be considered from the point of view of composition of fat provided by human milk and cow's milk. The higher levels of short-chain fatty acids in cow's milk fat have no significance in infant feeding. On the other hand, the differences in the levels of the unsaturated fatty acids in the two milks are of nutritional importance, human milk fat running higher than cow's milk fat.

The iodine number of the fatty acids of the blood serum of infants suffering from intractable eczema is lower than that of normal infants. Clinical improvement is noted, as well as a higher iodine number of the serum fatty acids, when unsaturated fatty acids are administered. Similar differences have been noted in the serum fatty acids of rats on low fat diets as compared with high.

Analyses of the serum fatty acids of healthy children from 3 to 14 yr. of age for two-, three-, and four-double bond fatty acids gave the following percentages: 30.3 ± 0.28, 1.5 ± 0.62, and 10.2 ± 1.44, respectively. Infant dietary intakes low in linoleic acid result in low serum di- and tetraenoic acids. The level of trienoic acid, on the other hand, is found high as compared with infants given adequate amounts of linoleic acid.

Similar results have been obtained on dogs. On a low fat intake, trienoic acid levels run high, while di- and tetraenoic acid levels are low. The reverse follows feeding dogs on an adequate fat diet. In the same experiment, the feeding of the ethyl or methyl esters of linoleic acid was less effective in raising the level of linoleic acid in the serum than the feeding of the triglyceride.

It has been observed in feeding a number of infants that a higher calorie intake is required to produce weight increase when a formula prepared from skimmilk is fed, compared with feeding a formula prepared from evaporated whole milk.

Experiments with a skimmilk formula supplemented with linoleic esters instead of fat indicate that the important calorie-sparing factor is linoleic acid. Whether the higher content of linoleic acid in human milk as compared with a formula prepared from whole cow's milk is important in infant health is yet to be determined. Frank E. Rice

246. Vitamin A deficiency in an infant. I. J. WOLF, Barnert Memorial Hosp. J. Am. Med. Assoc., **166**, 15: 1859. 1958.

This is a detailed report on the clinical and biochemical examinations at 3 and 5 mo. of an infant with an obvious vitamin A deficiency. During the mother's pregnancy she "abhorred milk and butter." Born prematurely (1,800 g.), the infant apparently had an extremely low store of vitamin A.

At 7 wk., because of an apparent intolerance to milk, the baby was placed on a soybean milk preparation. At the same time the physician instructed the mother to give the baby a vitamin preparation high in A, a routine which the mother failed to follow.

The classical picture of the vitamin A deficiency state, referable to the eyes and epithelial structures, was observed in this case. In addition there were signs, less well-known heretofore, referable to the nervous system, such as facial palsy, neck and back stiffness, and mental retardation. The infant responded well to vitamin A therapy. Frank E. Rice

247. Idiopathic hypercalcemia of infancy, with failure to thrive. A. M. BONGIOVANNI, W. R. EBERLEIN, AND I. T. JONES, Children's Hospital of Philadelphia and Univ. Penn. Sch.

of Med. New Eng. J. Med., **257**, Nov. 14: 951. 1957.

This is a detailed review of cases reported in medical literature, and a discussion of possible causes of idiopathic hypercalcemia, which may be a serious condition in infancy and may, in a few uncorrected cases, cause death. Reported also are three cases referred to the Philadelphia hospital in a 2-yr. period.

A hypersensitivity to vitamin D, with a history of feeding this ingestant at high levels, has been accepted as one of the incriminating factors. The incidence of the disease is greater in England where milk has been fortified with vitamin D to a level of 1,400 units per qt., than in the U.S.A, where fortification to the 400-unit level is the practice.

The fact that most babies can ingest equally high levels of vitamin D without untoward effects, indicates a specific sensitiveness to the vitamin in certain individuals.

The authors urge that in feeding the normal baby, the vitamin D intake be limited to from 400 to 500 I.U. per day. So many foods used in infant feeding other than milk are fortified with vitamin D in "our vitamin-hysterical environment" that it is sometimes difficult to determine the contributions of various hidden sources. Frank E. Rice

248. Vitamin B_6 in nutrition and metabolism. W. A. KREHL, Yale Univ., New Haven, Conn. Borden's Rev. of Nutrition Research, **18**, 5: 69. 1957.

Included in this comprehensive review is a statement of current knowledge of B_6 in milk products and in infant nutrition. The B_6 activity of fresh milk is due primarily to pyridoxal. Pasteurization causes no change. Heat-sterilization in the presence of amino acids and proteins converts part of the pyridoxal to pyridoxamine. The change also may continue during storage of the product. The pyridoxamine itself may be further converted to another unidentified form of vitamin B_6. Evaporated milk has long been used in infant nutrition without giving evidence of vitamin B_6 deficiency. Thus, it seems either that the human can utilize this unidentified form of vitamin B_6 or that evaporated milk contains enough of the known B_6 to meet the infants' requirements despite some conversion of the vitamin during processing. Frank E. Rice

249. Infant-feeding practices in hospital maternity nurseries. A survey of 1,904 hospitals involving 2,225,000 new-born infants. H. F. MEYER, Northwestern Univ. Med Sch., Chicago. Pediatrics, **21**, 2:288. 1958.

This is a report on a survey conducted in 1956 with hospitals having more than 300 births in 1955. The majority of hospitals, 82.1%, use a standard "house formula" in feeding all normal babies born there who are not completely breast fed. Of those hospitals reporting the practice of using a house formula, 75.4% used evaporated milk either with or without the addition of carbohydrate. The next most frequently used formulas were those made from commercial, premodified, prepared products. Many of the hospitals reported receiving these products free from the manufacturers. In a good many of these cases, on discharge from the hospital the babies were placed on a formula calling for milk, generally evaporated milk.

The use of pasteurized milk and "skimmed fresh milk" in making the house formulas was reported by 9% of the hospitals.

A surprisingly large number of hospitals, 11.5%, use formulas containing evaporated milk diluted with water, without the addition of carbohydrate. One large hospital consistently had used such a formula for more than 20 yr.

The age of the new-born infant at the time of discharge from the hospital varied from two to more than seven days; the largest number (46%) left the hospital at five days. During the stay in the hospital, 63% were completely bottle-fed, 16% breast- and bottle-fed, and 21% entirely breast-fed. There was no record of how long breast feeding was continued in the latter groups after leaving the hospitals. Frank E. Rice

250. Use of a formula diet for weight reduction of obese out-patients. A. R. FEINSTEIN, V. P. DOLE, AND I. L. SCHWARTZ, Rockefeller Inst. for Med. Research and N. Y. Univ. Coll. Med., New York City. Annals of Internal Med., **48**, 2: 330. 1958.

This is a report on 106 obese patients supervised through an out-patient clinic. Initial weights ranged from 147 to 380 lb. All passed through a brief training period, including physical examinations, before starting the prescribed diet. The diet consisted of a formula mixture containing 10 oz. evaporated milk, 6 level tbsp. dextrose, 2 level tbsp. corn oil, and 8 oz. water. This was the allowance for one day, to be divided into five or six feedings at intervals of 2 or 3 hr. The ingredients were chosen because they are readily available. Each day's allotment could be purchased in the New York City area for about $1.85. A multivitamin preparation was given which included mainly ascorbic acid and a number of the B vitamins. Unsweetened coffee and tea and low-calorie beverages were permitted.

The daily intake from the formula totals 900 calories, of which 10% is protein, 52% fat, and 38% carbohydrate. The outstanding advantages of the formula-feeding regime are its simplicity and inflexibility.

The patients reported to the clinic at weekly or bi-weekly intervals. Almost all patients found the formula palatable when cold and well-shaken. When desired, chocolate or other flavoring was added.

The results were varied—some patients departed from the formula early; others continued on the regime for over 21 wk. Fifty-three per cent lost 20 lb., 28% lost over 40 lb.

Many workers in sedentary or active occupations subsisted on 900 calories per day without serious fatigue. Some reported "weakness"; others claimed greater vigor than before. None showed signs of nutritional deficiency. Digestive disturbances and other symptoms varied with the individual. There were no psychic problems. On reaching target weights, most patients were instructed to change to normal diets gradually and, with care, their former objectionable habits were not resumed. The formula-feeding period is considered necessarily to be only the first stage of a reduction regimen.

Patients on this diet have maintained the same range of nitrogen balance as those on regular high-protein diets and have preserved normal values in all physiological variables tested. This formula regime is strikingly effective in certain instances where conventional weight-reducing plans have failed.

Frank E. Rice

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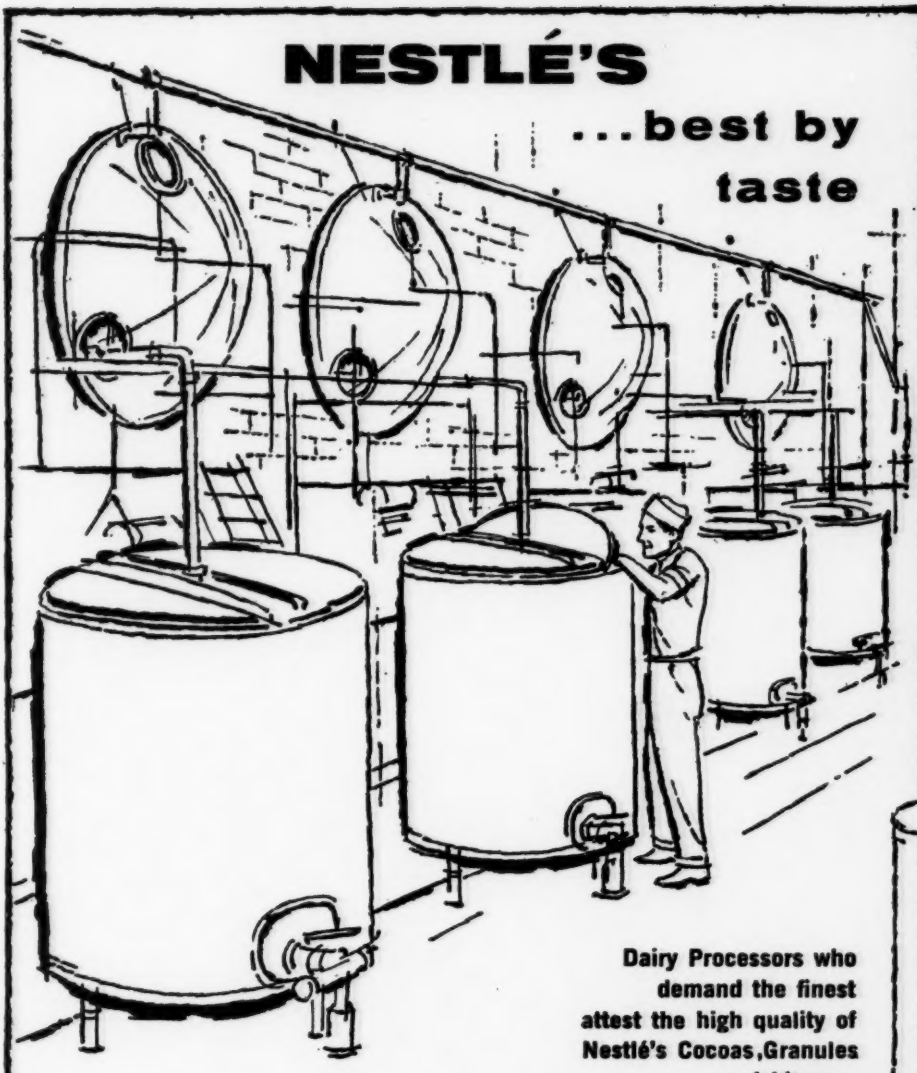

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